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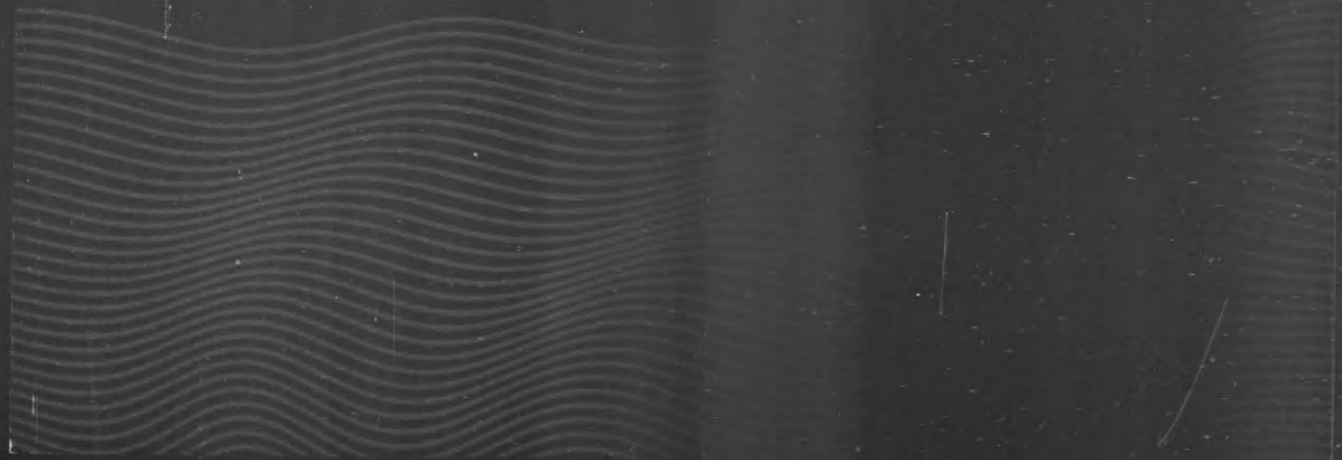
Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques



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Genetic Variation in Populations of Steelhead Trout (*Salmo gairdneri*) in British Columbia

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Parkinson, E. A. 1984. Genetic variation in populations of steelhead trout (*Salmo gairdneri*) in British Columbia. Can. J. Fish. Aquat. Sci. 41: 1412–1420.

The geographic distributions of biochemical genetic variants at four loci in indigenous British Columbia steelhead (*Salmo gairdneri*) populations indicated that there was a large-scale subdivision of the species into three major groups. At intermediate geographic scales, gene frequencies were uniform over unexpectedly large areas, but at the smallest geographic scale, significant differentiation was observed between populations in adjacent streams. This pattern of variation supports the view that this species is subdivided into a large number of semi-isolated populations each having the potential to evolve adaptations to local environmental conditions.

Les répartitions géographiques de variants génétiques pour les caractères biochimiques de quatre locus chez des populations indigènes de truite arc-en-ciel (*Salmo gairdneri*) de la Colombie-Britannique ont montré l'existence d'une subdivision, sur une grande échelle, de l'espèce en trois principaux groupes. Aux échelles géographiques intermédiaires, les fréquences des gènes étaient uniformes au sein de superficies beaucoup plus grandes que prévu tandis que l'on observait, à la plus petite échelle géographique, une différenciation significative entre des populations de cours d'eau adjacents. Cette allure des variations appuie l'hypothèse voulant que cette espèce soit subdivisée en un grand nombre de populations semi-isolées présentant toutes la capacité de s'adapter aux conditions locales du milieu.

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Genetic differentiation is now recognized as an important factor in the management of anadromous salmonids (Simon and Larkin 1972). Because of their migratory behavior and homing tendencies, differentiation in these species lies somewhere between the extremes of freshwater fish species, where extreme restriction of gene flow often leads to extensive differentiation between populations (Svardson 1979), and marine species, where physical barriers to gene flow are often absent and differences are often smaller (Carscadden and Misra 1980). Genetic variation in quantitatively inherited characteristics has been demonstrated among populations in a variety of anadromous salmonid species (Riddell et al. 1981; Refstie et al. 1977; Bams 1976; Raleigh 1971). In most cases, however, only a few populations were compared and it is difficult to generalize the results in terms of the prevalence of the differences and the degree of isolation necessary for differentiation.

In this report, the pattern of genetic differentiation in a large number of native populations of steelhead (the anadromous form of the rainbow trout, *Salmo gairdneri*) is examined using gene frequency data from five enzyme loci. Patterns of genetic differentiation in natural populations are more easily discernible using electrophoretic rather than quantitative data because a large number of populations can be sampled for characteristics that are not influenced by environmental factors. Surprisingly, there are few such studies on anadromous salmonids. Most authors have been interested in looking for major subdivisions within a species (Utter et al. 1970; Hodgins et al. 1969; Payne et al. 1971), in comparing specific populations or groups of populations (Busack et al. 1979; Aspinwall 1974), or in measuring genetic variability in the species as a whole using average heterozygosity of Wright's (1965) *F* statistics (Utter et al. 1973). In some cases (Moller 1971; Grant et al. 1980; Utter et al. 1970), the presence or absence of differences between nearby streams has been noted but no attempt was made to confirm the generality of the results. This study provides two pieces of information, the geographic distance at which steelhead populations can be shown to be genetically differentiated and the relative amount of genetic variation between populations in relation to distance between and the size of those populations.

Materials and Methods

A total of 150 samples comprising over 5000 individuals were collected from British Columbia streams (Table 1). Some locations were sampled repeatedly to test for differences in gene frequencies among year classes and among life history stages. Multiple samples from some streams were used to test for differences among locations more than 1 km apart. Although most samples consisted of fry or parr, some smolt and adult samples were also taken. Fry and parr were easily differentiated by size, but parr were not aged and probably contained at least two age groups. Smolts were captured in traps or gill nets and

were differentiated from parr by their size (>140 mm) and slim, silvery appearance. Adults were obtained from angler catches and hatcheries that utilized wild adults as brood stock. Samples were usually frozen on dry ice or brought back alive and immediately frozen. Some samples were preserved on ice for up to 12 h before being frozen.

Groups of fish frozen in a single block were thawed and then each fish was refrozen to facilitate removal of livers. Tissue extracts of liver and muscle were prepared by combining approximately equal amounts of tissue and distilled water in a 23 × 75 mm test tube, freezing this mixture for up to a week, and then centrifuging the samples at 100 × *g* for 5 min before loading the gel. Test tubes were kept immersed in ice water during sample preparation and gel loading. Centrifuging, homogenizing, and repeated freezing of the sample had no noticeable effect on the intensity or quality of the banding patterns of the enzymes used in this study.

Electrophoretic analyses were conducted using equipment and methods described by May et al. (1979). Two buffer systems were used. The first, after Ridgeway et al. (1970), electrode buffer 0.06 *M* LiOH (*M* = mol/L), 0.3 *M* boric acid (pH 8.3), gel buffer, 0.005 *M* citric acid, 0.03 *M* Tris (pH 8.3), was used for LDH and SOD. The second, after Shaw and Prasad (1970), 0.155 *M* Tris, 0.043 *M* citric acid (pH 7.0), full strength for the electrode buffer and diluted 1:15 with distilled water for the gel buffer, was used for MDH, IDH, and AGP. Staining solutions were adapted from Allendorf (1975).

Streams were defined as continuous areas of juvenile steelhead habitat separated from other suitable areas by areas of poor juvenile habitat (such as the ocean, a very large river, or an estuary). Streams can therefore be considered to contain a continuous population that may, or may not, be subdivided.

The locations of the streams and the boundaries of the geographical areas at each level in hierarchical design are given in Fig. 1. The bottom level of this hierarchy is samples within streams. The second level is streams within the small groups outlined in Fig. 1. A small group is two to four streams that are adjacent to each other. The third level is small group within a larger group of streams. A large group is a loosely defined term for the large geographic subdivisions outlined within the coastal region (R1) of Fig. 1. Dividing lines between large groups were assigned in a somewhat arbitrary fashion but were generally meant to follow natural features that may obstruct gene flow. Although arbitrarily bounded, large groups and small groups represent the type of divisions that are often made for management purposes. The fourth level is large groups within regions. Regions were treated as a level because previous work indicated that there were large differences between regions in both electrophoretic gene frequencies (Huzyk and Tsuyuki 1974) and life history characteristics such as run timing and size at maturity. The study is unbalanced in two respects: region R1 contains many more populations than either R2 or R3. In addition, samples were difficult to obtain from remote locations and these were sampled much less intensively.

A serious problem in sampling juvenile *Salmo gairdneri* is distinguishing anadromous steelhead from nonanadromous rainbow trout. I tried to minimize this problem through careful choice of sampling sites. Samples were not taken near the lakes where progeny of lacustrine rainbow may have been rearing. In R2, samples were taken below areas where large concentrations of steelhead were known to spawn. In R3, only smolt samples were used and in R2, either a smolt or adult sample was available from each stream. In R1, where stream resident rainbow above impassable barriers typically mature at a small size (<200 mm), I checked all parr samples for maturing individuals, which would indicate the presence of a nonanadromous population. Although a number of maturing males were found, no maturing females were found. Males that mature without going to sea are present in many anadromous salmonid species (Schmidt and House 1979; Osterdahl 1969; Bohlin 1975; Robertson 1957).

A variety of statistical methods have been used for hierarchical analysis of electrophoretic data including inbreeding statistics (Wright 1965), gene diversity analysis (Chakraborty 1980), maximum likelihood analysis (Smouse and Ward 1978), a type of variance component analysis (Smith et al. 1978), the Shannon–Weiner information index (Lewontin 1972), and simple hierarchical ANOVA (Avise and Felly 1979). As Nei (1975) pointed out, many of these are related and I used only two, gene diversity analysis and ANOVA of the arcsine square root transform frequency of the most common allele. I performed parallel analyses, one for each locus, rather than a single analysis in order to look at differences between loci.

Results

All variants used in this study have been previously reported. Mendelian inheritance has been documented for liver LDH (Allendorf 1973), SOD (Cederbaum and Yoshida 1972), white muscle MDH (Bailey et al. 1970), liver IDH (Allendorf 1975; Ropers et al. 1973; Reinitz 1977), and white muscle AGP (Allendorf 1975). LDH, SOD, and AGP were diallelic loci and MDH and IDH each had four alleles. MDH-118 seldom occurred at a frequency of over 0.05, while MDH-67 and MDH-72 were not initially distinguished, effectively making MDH a diallelic locus. The ANOVA only utilized data on the most common allele at each locus whereas the gene diversity analysis used data on two alleles for LDH, SOD, and MDH and four alleles for IDH. IDH data were missing for a number of samples where the initial run produced a poorly resolved pattern and insufficient tissue was available for a repeat run.

In two loci (LDH and SOD) the observed distribution of genotypes in each sample were compared with those expected from the assumption of Hardy–Weinberg equilibrium (*G*-test; Sokal and Rohlf 1981). The other two systems (MDH and IDH) consisted of pairs of loci and therefore, expected numbers of each genotype could not be generated. Only samples in which the expected number in the smallest class was greater than 4 were tested, a total of 47 samples for SOD and 8 samples for LDH. The sum of the individual *G*'s for LDH was not significant. The sum of the individual *G*'s for SOD was significant at $p < 0.5$, indicating a significant departure from Hardy–Weinberg expectations. There was no consistent excess or deficiency of heterozygotes; however, 25 samples had an excess and 22 samples had a deficiency. Samples of smolts and adults were taken when they were migrating and, therefore, are made up of a mixture of populations from different areas of the same stream. These showed no deviation from Hardy–Weinberg expectations. Of the 7 samples, 3 had an excess of heterozygotes and 4 had a deficiency.

Within-Stream Variation

There was significant variation among repeat samples within streams at all four loci (*G*-test, Sokal and Rohlf 1981). Three possible sources of this variation were taken into account: life

TABLE 1. Allele frequencies at five polymorphic loci in British Columbia steelhead populations. Only the frequency of the most common allele is given for LDH, SOD, MDH, and AGP. Locations of streams can be determined using the number in Fig. 1. No. samples refers to the number of repeat samples taken in each stream.

No.	Name	No. samples	LDH		SOD		MDH		IDH					AGP	
			N	Freq.	*N*	Freq.	*N*	Freq.	*N*	Freq.				*N*	Freq.
1	San Juan	1	40	1.000	40	0.550	39	0.859	38	0.724	0.079	0.171	0.026	40	1.000
2	Sarita	1	40	1.000	40	0.750	41	0.768	36	0.669	0.162	0.128	0.041	40	0.978
3	China	2	75	0.987	73	0.452	74	0.852	35	0.614	0.179	0.164	0.043	75	1.000
4	Stamp	2	67	0.985	54	0.658	76	0.928	36	0.618	0.208	0.153	0.021	76	1.000
5	Gold	3	126	0.929	120	0.667	126	0.909	86	0.712	0.125	0.139	0.023	99	0.995
6	Keogh	4	169	0.995	125	0.760	162	0.796	111	0.681	0.141	0.169	0.009	80	0.969
7	Tsulquate	1	30	0.933	17	0.794	23	0.783	13	0.654	0.135	0.077	0.135	9	1.000
8	Quatse	1	28	0.946	14	0.731	18	0.750	17	0.735	0.162	0.103			
9	Dean	1	45	1.000	42	0.821	26	0.981	32	0.664	0.164	0.047	0.125	23	1.000
10	Atnarko	1	72	0.986	66	0.727	72	0.924	54	0.597	0.282	0.056	0.065	58	0.940
11	Sooke	2	71	0.923	69	0.601	71	0.859	31	0.694	0.153	0.129	0.024	31	0.968
12	Kirby	2	21	0.810	24	0.542	59	0.686	20	0.700	0.150	0.075	0.075	61	1.000
13	Tugwell	2	76	0.882	71	0.725	106	0.670	68	0.698	0.184	0.114	0.004	98	0.985
14	Nanaimo	1	40	1.000	40	0.512	40	0.938	32	0.628	0.140	0.147	0.085	40	0.975
15	Cowichan	1	14	0.964	13	0.615	14	0.929						14	0.893
16	Englishman	2	50	0.980	40	0.475	46	0.794	26	0.615	0.192	0.163	0.029	18	0.972
17	French	1	51	0.990	50	0.580	51	0.902	48	0.714	0.177	0.026	0.083	51	1.000
18	Qualicum	2	129	0.942	116	0.634	112	0.844	87	0.650	0.144	0.124	0.083	126	0.964
19	L. Qualicum	2	76	0.947	61	0.639	76	0.829	40	0.650	0.162	0.150	0.037	76	1.000
20	Nile	2	69	0.993	65	0.808	74	0.824	36	0.528	0.285	0.174	0.014	74	1.000
21	Wilfred	2	75	1.000	72	0.430	74	0.865	36	0.674	0.146	0.125	0.056	38	0.947
22	Cowie	1	38	0.974	37	0.311	38	0.868	38	0.605	0.178	0.178	0.039	38	1.000
23	Rosewall	1	36	0.917	34	0.574	40	0.887	40	0.487	0.387	0.125		40	1.000
24	Headquarters	2	104	0.937	93	0.753	99	0.707	74	0.663	0.166	0.072	0.099	96	0.968
25	Trent	1	52	0.913	52	0.452	50	0.820	52	0.644	0.144	0.183	0.029	52	1.000
26	Puntledge	1	35	0.986	35	0.671	35	0.943	33	0.576	0.227	0.152	0.045	32	0.953
27	Quinsam	4	216	0.972	201	0.597	214	0.869	137	0.631	0.193	0.102	0.073	168	0.994
28	Oyster	1	26	1.000	12	0.542	25	0.900	16	0.641	0.156	0.156	0.047	23	0.957
29	Eve	1	27	1.000	29	0.897	31	0.919	19	0.684	0.171	0.145		32	0.969
30	Salmon	1	28	0.911	28	0.684	26	0.865	22	0.602	0.125	0.182	0.091	28	1.000
31	White	1	28	1.000	23	0.609	31	0.952	21	0.671	0.200	0.094	0.035	31	1.000
32	Cluxewe	1	22	0.977	21	0.833	32	0.984	16	0.719	0.125	0.156		35	0.985
33	Kokish	1	34	0.985	13	0.885	26	0.769	24	0.719	0.115	0.167		33	0.894
34	Nimpkish	1	34	0.985	30	0.733	26	0.846	26	0.538	0.375	0.038	0.048	30	0.917
35	Campbell	2	76	0.921	76	0.638	76	0.934	34	0.640	0.191	0.147	0.022	76	0.980
36	Bertrand	2	56	0.928	56	0.669	55	0.800	20	0.750	0.125	0.125		56	1.000
37	Seymour	2	62	0.935	52	0.740	61	0.836	50	0.675	0.075	0.200	0.050	57	1.000
38	Brohm	5	227	0.982	219	0.758	220	0.866	223	0.673	0.199	0.114	0.013	86	1.000
39	Stawamus	1	40	1.000	40	0.583	40	0.883	30	0.633	0.208	0.142	0.017	30	1.000
40	Squamish	1	37	0.973	34	0.794	40	0.887	35	0.700	0.129	0.171			
41	Wilson	1	36	1.000	34	0.382	37	0.932	31	0.573	0.250	0.097	0.081	32	1.000
42	Roberts	1	40	0.975	36	0.472	40	0.837	34	0.669	0.184	0.132	0.015	40	0.950
43	Chapman	1	40	1.000	39	0.449	40	0.900	38	0.566	0.191	0.191	0.053	40	1.000
44	Kleindale	1	46	0.880	12	0.583	46	0.891	36	0.618	0.118	0.125	0.139	8	0.938
45	Sliamon	1	39	1.000	38	0.421	39	0.897						36	0.986
46	Lang	1	8	1.000	8	0.438	8	0.875	8	0.625	0.250	0.031	0.094	8	1.000
47	Quatam	1	38	0.947	38	0.776	38	0.921						26	0.942
48	Apple	1	34	0.971	34	0.691	37	0.890	35	0.674	0.135	0.142	0.050	36	0.972
49	Kakweiken	1	35	1.000	35	0.786	35	0.814	33	0.689	0.265	0.038	0.008	35	0.971
50	Ahnuhati	1	25	1.000	26	0.792	27	0.722	20	0.775	0.112	0.112		27	0.963
51	Coquitlam	9	384	0.877	370	0.692	378	0.847	248	0.630	0.201	0.148	0.021	344	1.000
52	S. Alouette	7	261	0.912	260	0.619	260	0.952	169	0.667	0.219	0.096	0.18	205	0.990
53	N. Alouette	6	196	0.747	182	0.657	196	0.875	114	0.700	0.178	0.112	0.011	92	1.000
54	Salmon	4	231	0.994	195	0.728	230	0.798	171	0.649	0.199	0.136	0.016	203	0.983
55	Chilliwack	7	320	0.944	307	0.673	317	0.874	304	0.707	0.178	0.081	0.033	152	0.977
56	Weaver	7	293	0.969	288	0.477	284	0.518	158	0.618	0.263	0.089	0.030	290	1.000
57	Chehalis	4	141	0.855	135	0.582	140	0.911	72	0.682	0.201	0.090	0.028	70	0.979
58	Ruby	1	39	0.897	39	0.705	39	0.910	36	0.660	0.215	0.069	0.056	39	0.962
59	Coquihalla	2	51	0.784	51	0.844	51	0.951	49	0.658	0.151	0.145	0.046	51	0.961
60	Silverhope	1	40	0.837	40	0.587	40	0.850	40	0.631	0.188	0.087	0.094	40	0.975

TABLE 1. (*Concluded*)

No.	Name	No. samples	LDH		SOD		MDH		IDH					AGP	
			N	Freq.	*N*	Freq.	*N*	Freq.	*N*	Freq.				*N*	Freq.
61	Nahatlatch	5	138	0.909	138	0.750	137	0.960	88	0.625	0.196	0.128	0.052	111	0.964
62	Stein	1	36	0.681	36	1.000	30	0.883	8	0.688	0.188	0.125		36	0.958
63	Nicola	2	77	0.507	77	1.000	77	0.855	59	0.682	0.110	0.207		54	1.000
64	Thompson	1	50	0.440	50	1.000	49	0.857						50	0.980
65	Deadman	2	96	0.417	96	1.000	96	0.910	89	0.641	0.119	0.214	0.025	96	1.000
66	Bridge	2	66	0.531	66	1.000	59	0.788						64	0.890
67	Chilko	5	164	0.607	166	0.991	158	0.717	146	0.563	0.116	0.113	0.207	153	0.732
68	Lakelse	1	13	1.000	13	0.923	13	0.923	8	0.667	0.194	0.111	0.028	13	1.000
69	Zymoetz	1	22	0.977	22	0.909	22	1.000	21	0.631	0.143	0.190	0.36	22	1.000
70	Simpson	1	9	1.000	9	0.889	9	1.000	9	0.611	0.194	0.167	0.028	9	1.000
71	Deep	1	15	1.000	15	1.000	15	0.933	9	0.667	0.194	0.111	0.028	15	1.000
72	Kispiox	1	26	1.000	25	0.920	26	0.981	19	0.658	0.158	0.184		26	1.000
73	Babine	1	28	0.982	28	0.982	25	0.960	20	0.675	0.150	0.150	0.025	28	1.000

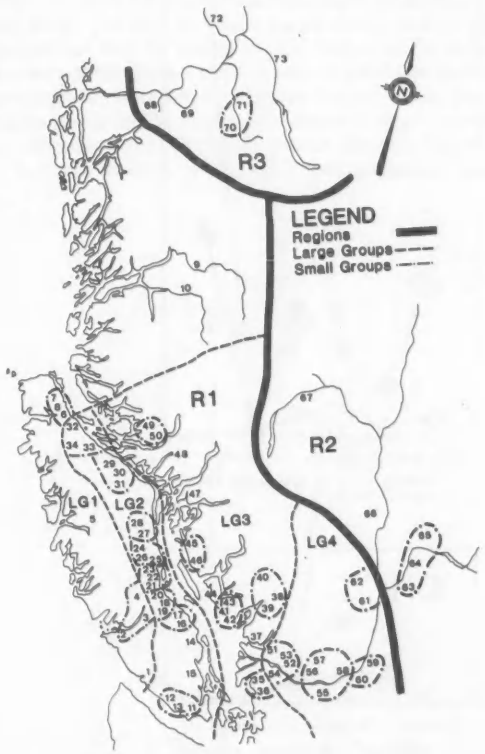

FIG. 1. Study area, including divisions between groups of streams at the various levels in the hierarchical analysis. Regions are labeled R1, R2, and R3 and the large groups within R1 are labeled LG1, LG2, LG3, and LG4. Numbers indicate the positions of streams and correspond to stream numbers in Table 1.

history stage, year classes, and locations within a stream. Four life history stages were considered: fry, parr, smolts, and adults. Samples of two or more of these stages were taken in the same year from the same rivers. Most comparisons (12 out of 15 degrees of freedom) were among samples of known steelhead (smolts or adults) and juvenile samples (fry or parr) where differences may indicate the presence of the progeny of nonanadromous rainbow trout. Year classes were compared by sampling fry at the same location in eight streams in two consecutive years. The differences accounted for by the three sources of variation are similar (Table 2). The absence of greater variability in life history stage comparisons indicates that either juvenile samples were not contaminated with nonanadromous fish or that any nonanadromous fish had gene frequencies similar to those of steelhead.

TABLE 2. Significance of variability between samples within streams (*$p < 0.05$; **$p < 0.01$; NS, not significant).

Source of variability	LDH	SOD	MDH	IDH	Total
Life history stage	NS	**	*	*	**
G	22.6	40.0	25.1	20.3	108.0
df	15	13	15	10	53
G/df					2.04
Year classes	NS	*	**	*	**
G	7.2	15.9	30.3	16.6	70.0
df	8	8	8	8	32
G/df					2.18
Locations	*	NS	**	NS	**
G	26.7	13.9	49.2	14.4	104.2
df	13	13	13	10	49
G/df					2.12

Among-Stream Variation

The ANOVA's indicate that variation is concentrated at the upper and lower ends of the hierarchy (Table 3). This pattern is consistent among the four loci with some exceptions. At the regional level the sharp cline between R2 and R1 at the LDH and SOD loci results in highly significant differences whereas much smaller differences among all three regions are present in MDH and IDH. At the large group level only LDH shows significant variation, a result of almost identical frequencies (0.962, 0.958, 0.963) in LG1, LG2, and LG3 and an unusually low frequency (0.891) in LG4, which is adjacent to R2 with a frequency of 0.537. A similar phenomenon, in which LG4 has a gene frequency intermediate between the interior region (R2) and the other coastal large groups, does not occur at the SOD locus. The

TABLE 3. Nested ANOVA's for each of the four enzyme systems. Numbers are the approximate F statistics with the corresponding degrees of freedom synthesized using the methods of Gaylor and Hopper (1969). $*p < 0.05$; $**p < 0.01$.

Source of variation	LDH	SOD	MDH	IDH
Regions	17.63 (2,4) **	46.05 (2,44) **	7.11 (2,44) **	3.25 (2,32) *
Large groups	4.00 (3,30) *	Pooled	Pooled	Pooled
Small groups	1.10 (30,40)	1.85 (30,40) *	1.19 (30,40)	1.14 (31,33)
Streams	3.66 (38,74) **	3.24 (38,75) **	1.62 (38,75) *	1.31 (36,53)

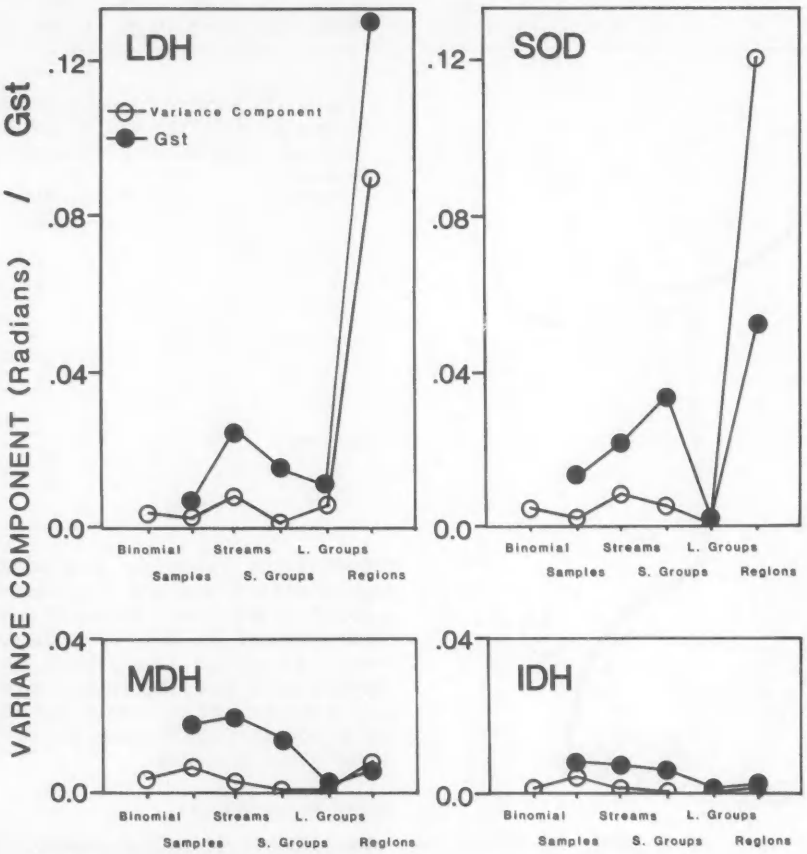

FIG. 2. Coefficients of gene differentiation from the gene diversity analysis and the corresponding variance components from the ANOVA analysis. Binominal variance components calculated by the method of Boygo and Becker (1965) are included for comparison.

variation at the lower levels cannot be associated with a few specific differences but is, instead, the result of a large number of small differences among components within a level. A simple correlation analysis of data from the three variable loci (LDH, SOD, MDH) was used to determine if large differences among streams within a small group at one locus were associated with large differences at other loci. None of the correlations were significant, indicating that the pattern of variation within small groups was not congruent among loci.

The pattern of variation between levels in the coefficients of gene differentiation from the gene diversity analysis and the corresponding variance components from the ANOVA analysis are similar, but there are some inconsistencies in the relative magnitude of components at different levels (Fig. 2). I have

ignored these differences and have assumed that the gene diversity analysis and other similar types of analysis (discussed by Nei 1975) would give similar results to the ANOVA.

AGP was essentially monomorphic except for one population, and a hierarchical analysis was not performed. The unusually low frequency (0.732) in this population (Chilko) is notable, however, since it is the population farthest upstream in R2. The sharp cline between Chilko and the rest of the R2 populations is important because it is at a different location from LDH and SOD clines, which coincide approximately with the R1–R2 boundary.

Cluster Analysis

Cluster analysis using Nei's (Nei and Roychoudhury 1974) genetic distance (Dm) in a UPGM analysis (Sneath and Sokal 1973) was used to complement the hierarchical analysis. When data from the four variable loci are used in a single analysis, five main clusters form, and there is a clear separation by regions (Fig. 3). The R2 populations form a single independent cluster and the R3 populations form a cluster that includes only three populations from the coastal region. Within the coastal region, however, there are four clusters, none of which are a continuous geographical unit. Smaller clusters formed within the coastal region at smaller Dm values also consist of populations that are not adjacent, but these clusters are not shown in Fig. 3.

A similar analysis of only the coastal populations was done using data for individual loci to generate four separate cluster maps. The purpose of this analysis was to determine if the lack of variation at the small and large group levels in the hierarchical analysis was due to the fact that the natural grouping of populations was ignored. At each locus a cut-off Dm was selected that would result in the separation of the 62 coastal populations into four clusters (Fig. 4). I felt that four clusters was the greatest number that could be depicted in an intelligible manner. The results reported here also apply to the smaller, more numerous clusters formed when smaller Dm values are used as separation criteria between clusters. There is little sign that the clusters formed by the UPGM analysis correspond in any way to a geographical grouping of populations at any of the four loci. The symbols in Fig. 4, especially those for larger clusters, tend to be scattered throughout the coastal region rather than being concentrated in a particular area. An exception is LDH where aberrant populations tend to cluster in one area. A significant variance component was present at the large group level at this locus. There is no evidence that the dividing lines at the small group and large group levels (Fig. 1 and 3) could have been judiciously altered to produce geographically cohesive, genetically similar clusters. If present, such clustering could have generated significant variance components at the large and small group levels if dividing lines were redrawn.

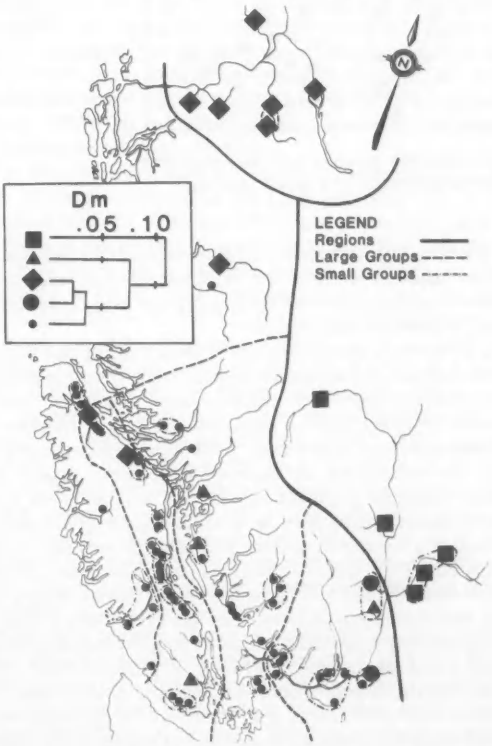

FIG. 3. Geographic locations of populations of the five principle clusters formed by a UPGM analysis of genetic distances (Dm) calculated using data from the four variable loci in a single analysis. The inset shows the actual distances between clusters.

Discussion

Genetic variation was present between steelhead populations in geographically adjacent streams, implying that gene flow is restricted over very short distances in this species. Some evidence for this was already available from studies of electrophoretic (Moller 1971; Campton 1981) and quantitative genetic (i.e. morphological, etc.) (Riddell et al. 1981; Refstie et al. 1977; Raleigh 1971) variation, as well as data on straying rates between populations of a number of anadromous salmonid species (Shapovalov and Taft 1954). Besides providing data specific to steelhead, I have strengthened and generalized this conclusion by comparing within- and between-population variability, by apportioning variation in a hierarchical manner, and by including a large number of natural populations in the analysis.

In electrophoretic studies interpopulation variability is often compared with a binomial sampling variance using a χ^2 test because repeat samples are required to estimate the true interpopulation variability. The implicit assumption in this procedure is that the gene frequency for a population is a constant. Significant variation in gene frequencies within populations invalidates the use of the χ^2 test because the apparent differences between populations may simply be due to real variation within populations. Differences were present at all four loci between repeat samples of steelhead populations from a single stream. The cause of these differences within populations is unknown but may be due to drift because the density of spawning adults is low (20–50/km) and the dispersal distances of juveniles are also low (<1 km). As a result, juveniles sampled at a single point may be the progeny of a limited number of parents and therefore have gene frequencies that differ significantly from the mean of the population in the stream as a whole. A similar result and possible explanation has been reported in anadromous cutthroat trout (*Salmo clarki clarki*) (Campton 1981), a closely related species that inhabits somewhat smaller streams than steelhead. In contrast with these results, a lack of within-stream heterogeneity has been reported

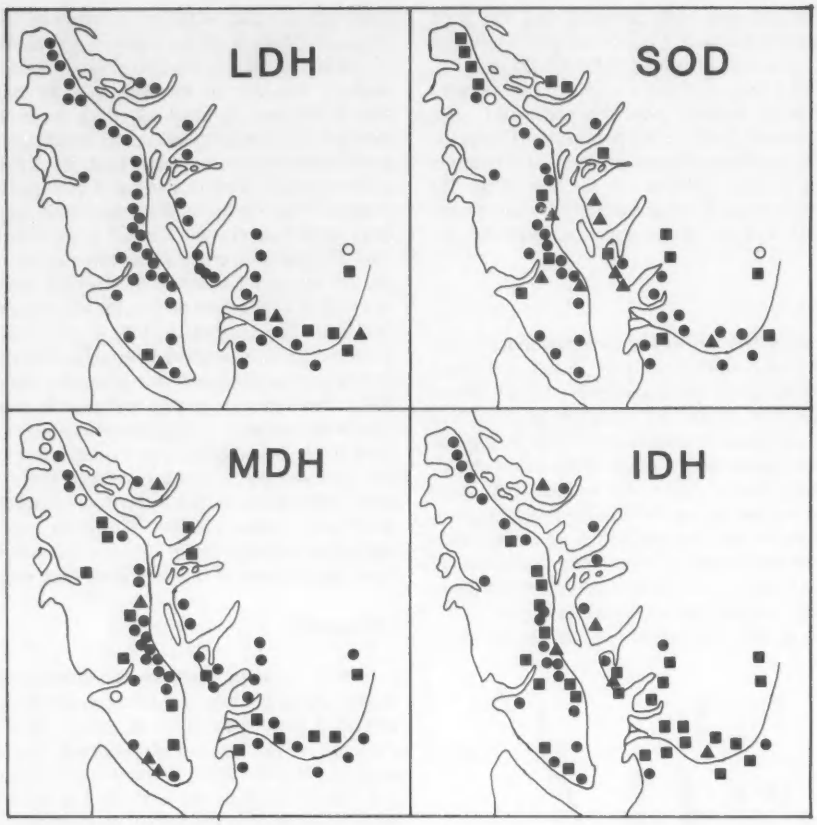

FIG. 4. Clusters of populations formed by UPGM analysis of genetic distances between populations calculated for individual loci. Populations within each cluster are identified by a unique symbol.

by Utter et al. (1980) for pink salmon (*Oncorhynchus gorbuscha*) and steelhead and by Grant et al. (1980) for sockeye salmon (*Oncorhynchus nerka*).

The hierarchical apportionment of variation was important in understanding the pattern of genetic differentiation in this species. The fish within a stream were assumed to be the basic unit of population structure, and samples within streams were used to measure the variation within these units. The possibility of stable genetic structuring within a stream was not examined. A simple one-level ANOVA of the differences between all streams would have detected the variation in the species as a whole but would have obscured the fact that a significant proportion of the variability within the species is associated with differences between streams that are very close together. Electrophoretic variation has been examined in a number of anadromous salmonid species including steelhead. Extensive variation between populations is common in most studies but, without a hierarchical analysis, variation cannot be related to the distances between and sizes of the populations involved.

The ANOVA and gene diversity analysis provide essentially the same pattern of results. Differences are significant at the adjacent stream level in three out of four loci and in one out of four loci at each of the next two levels. At the regional level there are very large differences in two out of four systems, which contrast with the smaller differences present in the other two systems. This pattern of variation implies that differences between adjacent streams are of approximately the same magnitude as those between streams that are many miles apart but still within the same region.

The differences among adjacent streams indicate that migration between such streams is not large enough to result in genetic homogeneity at these loci. Differentiation cannot be clearly attributed to either drift or selection. Both drift (Aspinwall 1974) and selection (Winter et al. 1980) have been implicated as causes for differences in electrophoretic gene frequencies between salmonid populations. Genetic differentiation over distances much smaller than the potential dispersal distance is common in a variety of species including *Drosophila affinis* (Richmond 1978), humans (Workman and Niswander 1970), *Mytilus edulis* (Koehn 1978), the red-bellied newts (Hedgecock 1978), and the eelpout (Christiansen and Frydenberg 1974).

As shown by the correlation analysis large differences among streams occur at different locations in different loci. This implies that the among-stream variance was not the result of situations where differences at all three variable loci coincided. I would suggest that any additional loci examined in the future will provide evidence of additional differentiation among adjacent streams rather than confirming the presently known pattern.

The apparent uniformity among large and small groups may

be the result of a number of factors. Stabilizing selection may be maintaining similar gene frequencies over a wide area. Secondly, the recent origin of these populations (since the last glaciation) may mean that differentiation has not had time to take place as yet, i.e. gene frequencies have not reached equilibrium values. Finally, the arbitrary boundaries may not coincide with the natural boundaries of clusters of steelhead populations. Cluster analysis of individual populations within a region reveals no natural clustering in populations on a geographical basis at any of the four loci, and boundaries could not have been redrawn to increase differentiation at these levels. Clustering by habitat type was not performed because of the difficulty in defining clear habitat categories. There is no way of distinguishing between the first two possibilities. Nevertheless, there is a trend to uniformity over very large distances in spite of the apparent lake of gene flow over very small distances. This trend is confirmed by the similarity of gene frequencies in northwestern United States (Allendorf 1975) and British Columbia coastal and interior steelhead populations:

	Coastal		Interior	
	B.C.(R1)	U.S.	B.C.(R2)	U.S.
LDH	0.931	0.874	0.471	0.433
SOD	0.646	0.663	0.996	0.930
MDH	0.870	0.886	0.862	0.985
IDH	0.690	0.660	0.696	0.676

Homogeneity over large distances coupled with heterogeneity on smaller geographic scales has been used by a number of authors in arguing for the presence of stabilizing selection at various loci in a number of species (Ayala et al. 1974; Christiansen and Frydenberg 1974; Richmond 1978; McKechnie et al. 1975). These arguments have some validity but cannot be regarded as conclusive evidence.

The sharp cline between the coastal and interior regions had been previously reported at the LDH and SOD loci on the Columbia River by Allendorf (1975) and at the LDH locus on the Fraser River by Huzyk and Tsuyuki (1974). Sharp clines, especially where more than one locus is involved, are often considered to be of major taxonomic significance. Allendorf (1975) and Child et al. (1976) have argued that these clines in steelhead and Atlantic salmon (*Salmo salar*), respectively, represent the present boundary between races that invaded newly available freshwater habitat following the last ice age. This argument is weakened in the case of steelhead by the absence of sharp clines in two loci (MDH, IDH) and the presence of a cline in another locus (AGP) at a different location. In addition, the Skeena River (R3) populations did not fit into either the interior (R2) or coastal (R1) group of populations. Differences, even major differences, at a small number of loci should not be taken to represent a fundamental subdivision of a species without corroborating evidence from a wider range of characteristics.

Differences between adjacent streams indicate that gene flow in this species is small over short distances. Ehrlich and Raven (1969) have argued that, in many species, natural selection is more important than gene flow in maintaining the similarity between populations of a species. This seems to be the situation in steelhead, a species that appears to be subdivided into a large number of semi-isolated demes that differ genetically from each other to varying degrees but still maintain a basic morphological and biological similarity that warrants their inclusion in a single species. With this type of population structure we should not expect that all of the differences we observe between populations are important adaptations to local environmental conditions. Many of these differences may be the result of weak selection or drift and therefore will not greatly affect fitness under various environmental conditions. Some differences may be more important but an important adaptive difference cannot be identified by simple observation even when the cause of the differentiation seems obvious.

The pattern of genetic variation reported here reflects an underlying stock structure that has to be considered in the management of this species. The presence of differences between adjacent streams supports the conclusion of straying, and genetic studies in various anadromous salmonid species which indicate that little interchange of individuals takes place between adjacent streams. Adjacent populations should therefore be managed as separate stocks.

Patterns of genetic variation are also important in situations, such as the transplantation of stocks, where variation itself has to be considered. Electrophoretic data provide some information on the presence of variation but none on its importance in terms of the relative fitness of populations in various environments. It should be noted that a similar situation exists in studies of genetic differences in morphology, behavior, or physiology even though the reasons for the differences in these cases may be more obvious. Information on the importance of genetic differentiation is unlikely to be available on a large scale in the near future because of difficulties in measuring the fitness of a large number of stocks in a large number of environments. Fisheries management decisions will, therefore, have to be made in the absence of this type of information. The most important information in this study is the scale at which differentiation can occur. Electrophoretic differentiation between adjacent populations implies that adaptive differentiation may occur on a small geographic scale. Stabilizing selection may, however, maintain similar phenotypes over a wider area. Since adjacent stocks may differ and widely separated stocks may be similar, the geographic distance between two stocks cannot be considered to be a measure of their genetic similarity and is, therefore, not very useful in assessing either the suitability of stocks for transplantation or the risk of disrupting genetic structuring through transplantation.

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Problems with Samples of Sea Lamprey (*Petromyzon marinus*) Ammocoetes Used in Stock Identification Studies[1]

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Allelic variation in sea lamprey (*Petromyzon marinus*) ammocoetes from the Lake Huron, Lake Michigan, and Lake Superior drainage basins was studied by starch gel electrophoresis. Previously untried enzyme systems were examined in sea lamprey ammocoetes. No new polymorphic enzyme systems were found. The allelic frequencies of ammocoete samples frequently failed to conform to Hardy–Weinberg equilibrium values. Allelic frequency variation observed among samples from the same drainage was larger than the variation observed among drainages. The results of this study and field observations suggest that the effective size of spawning groups that contribute progeny to samples is often small. These factors make ammocoete samples undesirable for use in stock identification studies.

La variation allélique d'ammocètes de la lamproie marine (*Petromyzon marinus*) des bassins hydrographiques des lacs Huron, Michigan et Supérieur a été étudiée par électrophorèse sur gel d'amidon. On s'est intéressé à des systèmes enzymatiques qui n'avaient encore jamais fait l'objet d'essai. Aucun nouveau système enzymatique polymorphe n'a été découvert. Les fréquences alléliques des échantillons d'ammocètes différaient fréquemment des valeurs à l'équilibre de Hardy–Weinberg. La variation des fréquences alléliques notée chez les échantillons d'un même bassin hydrographique était plus importante que celle notée entre les bassins. Les résultats de la présente étude et les observations faites sur le terrain indiquent que la taille effective des groupes de frai dont la progéniture se retrouve dans les échantillons est souvent petite. Tous ces facteurs font que les échantillons d'ammocètes sont inappropriés aux études d'identification des stocks.

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Electrophoretic investigations of genetic variation in sea lamprey (*Petromyzon marinus*) may be useful in the identification of sea lamprey stocks. Krueger (1980) found differences in the allelic frequencies of ammocoetes (larval sea lamprey) from the Lake Superior, Oneida Lake, Lake Champlain, and Bay of Fundy drainages. Krueger and Spangler (1981) studied allelic variation in sea lamprey ammocoetes from drainages in the Lake Superior basin and concluded that sea lamprey in Lake Superior may be subdivided into five discrete stocks. Brussard et al. (1981) found evidence for three genetically distinct groups of sea lamprey in eastern North America. Identification of sea lamprey stocks could have important implications for the control of this predator in the Great Lakes (Krueger 1980; Krueger and Spangler 1981).

In all previous studies a single sample of ammocoetes was generally used to estimate the allelic frequencies of all the ammocoetes in a drainage. Comparisons among drainages were made on the basis of these estimates. There are two potential problems with this approach. First, a single sample of ammocoetes may be inadequate for estimating the allelic frequencies of all the ammocoetes in a drainage. Second, statistical comparisons of the allelic frequencies of ammocoetes may give misleading results.

Single samples of ammocoetes are adequate for estimating the allelic frequencies of all the ammocoetes in a drainage only if variation among ammocoete samples from different locations in the same drainage is small relative to the variation among drainages. There is no a priori reason to believe that the variation among collections from the same drainage is small. Allelic variation that has been observed among single ammocoete samples from different drainages may be confounded by variation within drainages.

Tests for allelic heterogeneity among samples of ammocoetes have been used in previous studies as tests for heterogeneity among the spawning groups that produced the ammocoetes. It is

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the allelic variation among adults that is of interest in stock identification studies. Ammocoetes, rather than adults, are sampled only because they are convenient. Allendorf and Phelps (1981) discussed a problem with this approach. If the number of ammocoetes in a sample is larger than the number of spawners that produced the ammocoetes, then the degrees of freedom used to test for allelic heterogeneity are inflated and the probability of rejecting the null hypothesis of allelic homogeneity among spawning groups is inflated as well. Allendorf and Phelps (1981) warned that "Significant heterogeneity may be generated by our sampling scheme, rather than by isolation among subpopulations."

The purpose of the present study was to identify problems associated with the use of ammocoete samples in stock identification studies. In view of the potential importance of stock identification to sea lamprey control, these problems deserve serious consideration. The results of this study have bearing on the design of future investigations and the interpretation of previous ones.

Materials and Methods

General

Ammocoetes were collected by sea lamprey control personnel during 1982 and 1983 from tributaries of the upper Great Lakes by electroshocking or by application of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) as described by Smith et al. (1974). Drainages sampled were among those visited by sea lamprey control personnel in the course of their regular duties. Sampling locations within individual drainages were chosen for convenience. Ammocoetes collected at each location were frozen as quickly as possible, transported to the laboratory, and stored at −20°C until they were analyzed.

Variation in the expression of enzyme loci was studied by horizontal starch gel electrophoresis as described by May et al. (1979). Four polymorphic enzyme loci described by Krueger (1980) were used: *AGP*, alpha-glycerophosphate dehydrogenase (EC 1.1.1.8); *MDH-1*, malate dehydrogenase (EC 1.1.1.37); *PGI-2*, phosphoglucose isomerase (EC 5.3.1.8); *PGM-1*, phosphoglucomutase (EC 2.7.5.1). The nomenclature for enzymes and enzyme loci used here is that of Krueger (1980) and Allendorf and Utter (1979). Buffer systems, sample preparation, and enzyme stains used were the same as those described by Krueger (1980) except that a Tris–citrate buffer system described by Siciliano and Shaw (1976) was used for AGP and PGI-2 because it improved the resolution of these enzymes.

Enzyme Screening

Enzyme systems that had not been previously examined were screened for polymorphic expression in sea lamprey ammocoetes. The activity and resolution of the following enzymes were examined in a preliminary screening: acid phosphatase (EC 3.1.3.2), alpha-galactosidase (EC 3.2.1.22), alpha-mannosidase (EC 3.2.1.24), beta-glucosidase (EC 3.2.1.21), beta-glucuronidase (EC 3.2.1.31), glutamate-pyruvate transaminase (EC 2.6.1.2), glycerate 2-dehydrogenase (EC 1.1.1.29), nucleoside phosphorylase (EC 2.4.2.1), and 2,3-phosphoglycerate mutase (EC 5.4.2.1). Recipes for enzyme stains were taken from Siciliano and Shaw (1976). Each stain was tested on four buffer systems: Ridgway and Clayton–Tretiak buffer systems described by Krueger (1980) and the Tris–citrate and Tris–versene–borate systems described by Siciliano and Shaw (1976).

Five enzymes showed good activity and good resolution: alpha-mannosidase, beta-glucosidase, beta-glucuronidase, and glutamate-pyruvate transaminase on the Tris–versene–borate buffer system; acid phosphatase on the Clayton–Tretiak buffer system. The banding patterns for each of these enzymes were examined in a total of 200 ammocoetes from several Lake Superior and Lake Michigan drainages.

Statistical Analyses

Analyses were based on a total of 40 ammocoete samples. Samples from four locations in each of four Lake Michigan drainages, from two locations in each of six Lake Huron drainages, and from three locations in each of four Lake Superior drainages were used (Fig. 1).

Genotypic frequencies for each sample were tested for conformance to Hardy–Weinberg equilibrium values by an exact test (Haldane 1954) as described by Elston and Forthofer (1977). Probability values smaller than or equal to 0.05 were required for rejection of the null hypothesis of conformance to the Hardy–Weinberg model. Five percent of these tests should, on average, reject the null hypothesis. If more than 5% of the individual tests reject the null hypothesis, then there is evidence that genotypic frequencies for the locus do not generally conform to the Hardy–Weinberg model.

The total variance in the allelic frequencies of ammocoete samples from a lake was partitioned into within- and between-drainage components by a hierarchical analysis of variance described by Wright (1978, p. 86–89) using the computer program BIOSYS-1 (Swofford and Selander 1981). Sample sizes were balanced for hierarchical analyses so that calculation of variance components would be simplified (Sokal and Rohlf 1981, p. 293). After electrophoresis, data for 35 ammocoetes were selected from the total for each of the Lake Michigan sampling locations. Data for 50 and 33 ammocoetes were selected, respectively, from the total for Lake Huron and Lake Superior sampling locations. This was done by excluding all individuals for which the genotype at one more loci could not be determined (because of poor sample condition or small size of an ammocoete) and then sampling randomly until 35, 50, or 33 individuals remained.

Results and Discussion

No polymorphisms were detected in the enzymes that were screened during this study. The rest of this report deals with variation in the four polymorphic enzymes described previously (*AGP*, *MDH-1*, *PGI-2*, and *PGM-1*).

Genotypic Frequencies

Genotypic frequencies (Table 1) did not conform to Hardy–Weinberg equilibrium values as often as expected on the basis of chance. Genotypic frequencies of Lake Michigan samples differed significantly from Hardy–Weinberg values in 8 of 64 (12.5%) of the total comparisons: Platte River 1, *AGP* ($F = -0.387$); Lincoln River 2, *AGP* ($F = -0.347$); Lincoln River 3, *AGP* ($F = -0.357$); Lincoln River 4, *PGM-1* ($F = -0.382$); Pere Marquette River 1, *MDH-1* ($F = 0.389$); Pere Marquette River 2, *MDH-1* ($F = 0.195$); Pere Marquette River 4, *PGM-1* ($F = -0.326$); Cedar River 3, *PGM-1* ($F = -0.245$). The *F*-statistic, calculated from observed and expected frequencies

FIG. 1. Drainages from which ammocoetes were collected.

of heterozygotes in samples (Nei 1977), is used to describe the departure of a genotypic distribution from Hardy–Weinberg values. Positive values of the statistic indicate heterozygote deficiencies, and negative values indicate heterozygote excesses. Values of the statistic may range between −1 and 1, and a value of zero indicates perfect conformance to Hardy–Weinberg equilibrium values.

Genotypic frequencies of Lake Huron samples differed significantly from Hardy–Weinberg equilibrium values in 5 of 48 (10%) of the total comparisons: Ocqueoc River 1, *AGP* ($F = -0.414$); Ocqueoc River 2, *MDH-1* ($F = 0.318$); Rifle River 1, *PGI-2* ($F = -0.505$) and *PGM-1* ($F = 0.909$); Gordons Creek 2, *AGP* ($F = 0.550$).

Genotypic frequencies of Lake Superior samples differed significantly from Hardy–Weinberg equilibrium values in 4 of 48 (8%) of the total comparisons: Brule River 1, *PGI-2* ($F = 1.00$); Little Two Hearted River 3, *MDH-1* ($F = 0.791$); Two Hearted River 1, *MDH-1* ($F = -0.299$); Two Hearted River 2, *AGP* ($F = 0.289$).

The *F*-statistics for the genotypic distributions that did not conform to Hardy–Weinberg expectations were generally of large absolute value (>0.25), indicating substantial deviation from expected values. The *F*-statistics were not consistently positive or consistently negative, indicating that both heterozygote deficiencies and excesses were common.

Brussard et al. (1981), Krueger (1980), and Krueger and Spangler (1981) reported that the genotypic frequencies of ammocoete samples differed from Hardy–Weinberg equilibrium values in 4 of 60, 0 of 19, and 3 of 72 cases, respectively. In the present study, 17 of 160 cases differed from Hardy–Weinberg values. These proportions are not significantly different from one another (chi-square test, $p = 0.18$) and can be combined to estimate the overall frequency of deviation from Hardy–Weinberg equilibrium values. Overall, 24 of 311 (8%) genotypic distributions differed significantly from Hardy–Weinberg equilibrium values. A tendency to diverge from Hardy–Weinberg equilibrium values appears to be characteristic of genotypic frequencies of ammocoete samples. This characteristic may have gone undetected in previous studies because relatively few samples were used.

Genotypic frequencies of ammocoete samples may fail to conform to Hardy–Weinberg equilibrium values for a number of reasons (Falconer 1981, p. 11–13). A single sample of ammocoetes may contain three or more year-classes depending on when the drainage was last subjected to a sea lamprey control treatment, and the rate at which the drainage was recolonized by sea lamprey (Manion and Smith 1978). A Wahlund effect (Hartl 1980, p. 191–195) due to mixing of year-classes with different allelic frequencies may account for the heterozygote deficiencies observed in some samples. Small effective size of spawning populations (discussed below) may account for the heterozygote excesses observed. Another possible explanation is that deviations from Hardy–Weinberg equilibrium were observed because the enzyme polymorphisms do not have a simple genetic interpretation. Mendelian inheritance of the enzyme systems used here has not been verified for sea lamprey.

Allelic Variation within and among Drainages

Allelic variation among samples of ammocoetes from the same drainage was larger than the variation among drainages (Table 2). Negative variance components with small absolute

TABLE 1. Genotypic frequencies, allelic frequencies, and sample sizes for ammocoete samples from tributaries to Lake Michigan, Lake Huron,

	AGP						*MDH-1*				
		Genotypes			Alleles			Genotypes			Alleles	
Sample	*N*	100/100	100/146	146/146	100	146	*N*	−100/−100	−100/−165	−165/−165	−100	−165
Lake Michigan												
Platte River												
1	40	12	26	2	0.63	0.37	40	33	6	1	0.90	0.10
2	37	8	21	8	0.50	0.50	38	30	8	0	0.90	0.10
3	39	21	16	2	0.74	0.26	39	20	18	1	0.74	0.26
4	40	16	22	2	0.67	0.33	40	23	17	0	0.79	0.21
Lincoln River												
1	47	10	20	17	0.43	0.57	48	43	5	0	0.95	0.05
2	59	14	39	6	0.57	0.43	59	48	11	0	0.91	0.09
3	57	0	30	27	0.26	0.74	57	57	0	0	1.00	0.00
4	57	13	27	17	0.47	0.53	57	44	13	0	0.89	0.11
Pere Marquette River												
1	55	20	27	8	0.61	0.39	55	45	7	3	0.88	0.12
2	118	27	68	23	0.52	0.48	122	87	28	7	0.83	0.17
3	57	35	19	3	0.78	0.22	57	40	17	0	0.85	0.15
4	56	33	23	0	0.80	0.20	57	34	23	0	0.80	0.20
Cedar River												
1	61	26	25	10	0.63	0.37	61	39	19	3	0.79	0.21
2	48	9	22	17	0.42	0.58	60	33	25	2	0.76	0.24
3	69	21	31	17	0.53	0.47	74	44	29	1	0.79	0.21
4	35	8	21	6	0.53	0.47	55	39	14	2	0.84	0.16
Lake Huron												
Ocqueoc River												
1	58	9	41	8	0.51	0.49	59	38	17	4	0.79	0.21
2	59	12	24	23	0.41	0.59	60	38	15	7	0.76	0.24
Rifle River												
1	51	5	26	20	0.35	0.65	51	50	1	0	0.99	0.01
2	51	14	24	13	0.51	0.49	51	38	11	2	0.85	0.15
Watson's Creek												
1	59	18	27	14	0.53	0.47	59	42	15	2	0.84	0.16
2	58	10	33	15	0.46	0.54	58	50	8	0	0.93	0.07
Gordon's Creek												
1	55	6	27	22	0.36	0.64	55	32	23	0	0.79	0.21
2	58	38	10	10	0.74	0.26	59	45	14	0	0.88	0.12
Blind River												
1	50	9	21	20	0.39	0.61	57	46	11	0	0.90	0.10
2	58	13	33	12	0.51	0.49	58	52	6	0	0.95	0.05
St. Mary's River												
1	58	18	26	14	0.53	0.47	60	44	14	2	0.85	0.15
2	59	16	33	10	0.55	0.45	59	37	20	2	0.80	0.20
Lake Superior												
Brule River												
1	52	30	18	4	0.75	0.25	58	39	17	2	0.82	0.18
2	62	12	36	14	0.48	0.52	62	44	18	0	0.85	0.15
3	59	22	23	14	0.57	0.43	60	48	10	2	0.88	0.12
Huron River												
1	53	22	24	7	0.64	0.36	59	43	16	0	0.86	0.14
2	65	27	29	9	0.64	0.36	67	39	26	2	0.78	0.22
3	65	30	27	8	0.67	0.33	66	45	21	0	0.84	0.16
Little Two Hearted River												
1	48	18	25	5	0.64	0.36	48	22	22	4	0.69	0.31
2	55	9	29	17	0.43	0.57	59	42	16	1	0.85	0.15
3	33	4	17	12	0.38	0.62	58	55	1	2	0.96	0.04
Two Hearted River												
1	56	36	18	2	0.80	0.20	57	23	32	2	0.68	0.32
2	48	17	17	14	0.53	0.47	50	35	11	4	0.81	0.19
3	50	18	21	11	0.57	0.43	50	32	16	2	0.80	0.20

and Lake Superior.

	PGI-2									*PGM-1*					
	Genotypes						Alleles				Genotypes			Alleles	
N	90/90	90/100	90/122	100/100	100/122	122/122	90	100	122	*N*	100/100	100/148	148/148	100	148
40	0	0	0	38	2	0	0.00	0.97	0.03	40	6	24	10	0.45	0.55
38	0	0	0	34	4	0	0.00	0.95	0.05	38	17	16	5	0.66	0.34
38	0	11	0	25	2	0	0.14	0.83	0.03	39	16	22	1	0.69	0.31
40	0	0	0	40	0	0	0.00	1.00	0.00	40	5	21	14	0.39	0.61
48	0	0	0	46	2	0	0.00	0.98	0.02	48	9	29	10	0.49	0.51
59	0	12	0	43	4	0	0.10	0.86	0.04	59	25	26	8	0.64	0.36
57	0	0	0	57	0	0	0.00	1.00	0.00	57	18	26	13	0.54	0.46
57	0	1	1	53	2	0	0.02	0.95	0.03	57	17	37	3	0.62	0.38
55	0	0	0	55	0	0	0.00	1.00	0.00	55	30	22	3	0.74	0.26
122	0	2	0	116	4	0	0.01	0.97	0.02	121	46	59	16	0.62	0.38
57	0	0	0	57	0	0	0.00	1.00	0.00	57	13	28	16	0.47	0.53
57	0	0	0	57	0	0	0.00	1.00	0.00	57	29	28	0	0.75	0.25
61	0	0	0	61	0	0	0.00	1.00	0.00	61	25	30	6	0.66	0.34
60	0	6	0	49	5	0	0.05	0.91	0.04	60	28	29	3	0.71	0.29
74	0	2	0	69	3	0	0.01	0.97	0.02	74	27	42	5	0.65	0.35
55	0	1	0	51	3	0	0.01	0.96	0.03	55	27	22	6	0.69	0.31
59	0	0	1	48	9	1	0.01	0.89	0.10	59	29	27	3	0.72	0.28
60	0	3	0	56	1	0	0.02	0.97	0.01	60	33	24	3	0.75	0.25
51	0	3	0	14	34	0	0.03	0.64	0.33	51	15	2	34	0.31	0.69
51	0	1	0	47	3	0	0.01	0.96	0.03	51	23	21	7	0.66	0.34
58	0	2	0	56	0	0	0.02	0.98	0.00	59	34	24	1	0.78	0.22
58	0	1	0	57	0	0	0.01	0.99	0.00	58	22	33	3	0.66	0.34
55	0	0	0	45	10	0	0.00	0.91	0.09	55	17	32	6	0.60	0.40
59	0	0	0	59	0	0	0.00	1.00	0.00	59	26	29	4	0.69	0.31
57	0	0	0	35	21	1	0.00	0.80	0.20	57	27	23	7	0.67	0.33
58	0	0	0	40	17	1	0.00	0.84	0.16	58	25	25	8	0.65	0.35
60	0	0	0	60	0	0	0.00	1.00	0.00	60	26	31	3	0.69	0.31
59	0	2	0	51	6	0	0.02	0.93	0.05	59	24	30	5	0.66	0.34
58	1	0	0	57	0	0	0.02	0.98	0.00	58	24	25	9	0.63	0.37
62	0	2	0	55	5	0	0.02	0.94	0.04	62	25	24	13	0.60	0.40
60	0	5	0	54	1	0	0.04	0.95	0.01	60	29	22	9	0.67	0.33
59	0	0	0	57	2	0	0.00	0.98	0.02	58	23	24	11	0.60	0.40
67	0	2	0	63	2	0	0.02	0.97	0.01	67	15	39	13	0.52	0.48
66	0	1	0	65	0	0	0.01	0.99	0.00	66	24	33	9	0.61	0.39
48	0	0	0	48	0	0	0.00	1.00	0.00	48	32	12	4	0.80	0.20
59	0	11	0	47	1	0	0.09	0.90	0.01	59	16	31	12	0.53	0.47
58	0	3	0	55	0	0	0.03	0.97	0.00	58	16	29	13	0.53	0.47
57	0	3	0	49	5	0	0.03	0.93	0.04	57	21	31	5	0.64	0.36
50	0	3	0	45	2	0	0.03	0.95	0.02	50	16	25	9	0.57	0.43
50	0	5	0	38	7	0	0.05	0.88	0.07	50	21	21	8	0.63	0.37

value were obtained in some cases. Small negative values were taken to be evidence that the true variance component was zero (Searle 1971, p. 406–408). The variance within and among drainages can be expressed as proportions of the total variance (Sokal and Rohlf 1981, p. 283). The weighted average of the variation in allelic frequencies within Lake Michigan drainages was 84% of the total variation. The weighted average of the variation within Lake Huron and Lake Superior drainages was 100% of the total.

These results show that a single sample of ammocoetes is inadequate for estimating allelic frequencies for all the ammocoetes in a tributary of the upper Great Lakes. Multiple sampling of individual drainages would be required to estimate the allelic frequencies of all the ammocoetes in a drainage.

Previous workers (Krueger 1980; Krueger and Spangler 1981; Brussard et al. 1981) observed allelic frequency variation among samples of ammocoetes from different drainages. In the present study there was no evidence of allelic variation among drainages of Lake Superior. There was evidence of relatively little variation among drainages of Lake Michigan and Lake Huron. Variation observed among drainages in previous studies may have been due, in large part, to the confounding effects of allelic variation within drainages. Results of this study suggest that the variation among samples within drainages overwhelms the variation among drainages.

All collections from the Lake Superior basin for the present study were taken along the south shore of the lake. Greater variation among drainages of the Lake Superior basin may have been observed if collections from other areas of the lake had been included.

TABLE 2: Variance components for sampling locations within drainages, and drainages within lakes. Variance components are expressed as percentages of the total variance. Variance due to sampling error was not included in the calculations. Negative variance components were taken to be zero. The values for individual loci were weighted by $p(1 - p)$ (where p is the frequency of the most common allele) and then averaged over loci to give the overall values.

Locus	Locations within drainages	Drainages within lakes	Total
Lake Michigan ($N = 35$)			
AGP	68	32	0.034
MDH-1	67	33	0.0086
PGI-2	100	0	0.0038
PGM-1	100	0	0.017
Overall	84	16	0.063
Lake Huron ($N = 50$)			
AGP	100	0	0.014
MDH-1	58	42	0.0062
PGI-2	88	12	0.019
PGM-1	100	0	0.020
Overall	100	0	0.059
Lake Superior ($N = 33$)			
AGP	100	0	0.021
MDH-1	100	0	0.0010
PGI-2	100	0	0.0015
PGM-1	100	0	0.00078
Overall	100	0	0.034

Effective Population Size

The results of this study suggest that the number of spawners contributing progeny to ammocoete samples (the effective population size of spawning groups) is often small. If the size of spawning groups is sufficiently small, then allelic frequency differences will arise between the parental sexes on the basis of chance. Robertson (1965) showed that heterozygote excesses will occur under these conditions. Heterozygote excesses are common in the data presented here.

Variation observed in this study among samples from different locations in the same drainage supports the possibility that the effective size of spawning groups is often small. The variation among ammocoete collections probably reflects variation in the allelic frequencies of spawning groups at different locations in the drainage. Variation in the allelic frequencies of spawning groups in a drainage will arise if the number of spawners in some of the groups is small. Variance in the allelic frequencies of local spawning groups of size n drawn from a large spawning run to a drainage is the binomial variance of sampling means $pq/2n$ where p and q are allelic frequencies (Falconer 1981, p. 49). Variance is inversely proportional to the size of the spawning groups so that the variance in allelic frequencies of spawning groups will increase as the size of the spawning groups decrease. If spawner abundance is low (due to the ongoing sea lamprey control) or spawning habitat is widely scattered, then the ammocoetes at a single location may be the progeny of few adults. The number of successful spawners will be further reduced by differences in spawning success, differences in survival among sibgroups (Manion and Hanson 1980), unbalanced sex ratios (Heinrich et al. 1980), and polygyny (Hanson and Manion 1978, p. 11).

Field observations support the hypothesis that the number of sea lamprey in spawning groups is often small. Catches of spawning run sea lamprey by electric weirs on index streams in the Lake Superior basin show that small runs of spawning sea lamprey are common, particularly since the advent of sea lamprey control (Smith et al. 1974).

The Pere Marquette River illustrates the possibility that spawning groups may be of small size. The Pere Marquette River is a large dendritic drainage with many miles of suitable spawning habitat that consistently support high levels of ammocoete production. The abundance and distribution of spawning habitat probably limits the number of spawning adults that utilize any particular spawning site. During June 9–25, 1971 (sea lamprey spawning season), U.S. Fish and Wildlife Service personnel examined 200 km of sea lamprey spawning habitat along the Pere Marquette River. No spawning lamprey and only 17 nests were observed. During May 4–June 22, 1973, 172 km of stream along the Pere Marquette River was examined; no spawning adults and only nine nests were observed (U.S. Fish and Wildlife Service, Ludington, MI, unpubl. data). Sea lamprey spawn in the daytime over a period of weeks (Applegate 1950) and would have been observed if present in large numbers.

The results of this investigation, along with field observations, suggest that the effective size of localized spawning groups of sea lamprey in the upper Great Lakes is often small. For this reason allelic frequency differences observed between ammocoete samples, even though statistically significant, should be regarded with caution.

Problems with ammocoete samples make them unsuitable for electrophoretic investigations of stock structure. A better approach in future stock identification studies would be to use

samples of spawning adults collected at the mouths of rivers during the spawning season.

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Efficiency of Large-Volume Plankton Pumps, and Evaluation of a Design Suitable for Deployment from Small Boats[1]

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Taggart, C. T., and W. C. Leggett. 1984. Efficiency of large-volume plankton pumps, and evaluation of a design suitable for deployment from small boats. Can. J. Fish. Aquat. Sci. 41: 1428–1435.

A critical review of previously published studies, and evaluation of a newly developed pump system, showed the often stated shortcomings of large-volume pumps as instruments for sampling plankton communities (e.g. damage to organisms sampled, volumes too small to estimate plankton density accurately, and systems too cumbersome to handle) to be invalid. Previously published estimates of the comparative efficiency of large-volume pump samplers and tow nets should, however, be viewed with caution because the techniques used to estimate pumped volume are error prone. In-line flow meters are essential for accurate volume estimation. Comparative efficiency tests of a newly developed large-volume pumping system deployed simultaneously with 80- and 153-μm mesh standard plankton nets revealed that the pump, while sampling only 8% of the volume sampled by the nets, was equally effective in capturing capelin (*Mallotus villosus*) larvae (5-mm length), herring (*Clupea harengus*) larvae (9-mm length), large copepods (>750 μm), small jellyfish, and hyperiid amphipods. The pump was superior to nets in sampling crab zoea and megalops larvae and becomes more efficient at capturing euphausiids and chaetognaths as their natural densities increase. Nets were superior in the capture of fish eggs (primarily cunner, *Tautogolabrus adspersus*), possibly due to the vertical distribution of eggs in the water column. The average length of capelin larvae captured by pumping was consistently 0.2 mm longer than that of larvae taken in nets, but the length–frequency distribution of larvae sampled was similar to that of larvae entering the pelagic environment. Attaching the pump intake to the cod-end of a rigid tow net is suggested as a method of surmounting potential errors when sampling organisms at densities below 1–5/m^3.

Un examen détaillé des publications et l'évaluation d'une pompe récemment mise au point ont révélé que les imperfections souvent mentionnées pour les pompes à grand volume servant à l'échantillonnage des communautés planctoniques (par ex. dommages aux organismes, volumes trop petits pour l'estimation précise des densités, appareillage trop encombrant) n'étaient pas fondées. Les estimations publiées de l'efficacité comparative de pompes d'échantillonnage à grand volume et de filets à plancton doivent être considérées avec prudence, les techniques utilisées pour l'estimation du volume pompé étant sujettes à erreur. Des débitmètres en ligne sont essentiels pour l'estimation précise du volume. Des tests de l'efficacité comparative d'une pompe à grand volume, récemment mise au point et déployée simultanément avec des filets à plancton standards à mailles de 80 et 153 μm, ont révélé que la pompe, même si elle ne recueillait que 8 % du volume échantillonné par les filets, était aussi efficace pour la capture de larves de capelan (*Mallotus villosus*, longueur : 5 mm) et de hareng (*Clupea harengus*, longueur : 9 mm), de gros copépodes (>750 μm), de petites méduses et d'amphipodes (Hyperiidae). La pompe s'est révélée supérieure aux filets pour l'échantillonnage des zoés et des mégalopes de crabe et plus efficace pour la capture des euphausiacés et des chétognathes à mesure que leur densité naturelle augmentait. Par contre, les filets se sont révélés supérieurs pour le prélèvement des œufs de poisson (surtout de la tanche-tautague *Tautogolabrus adspersus*), probablement à cause de la répartition verticale des œufs. La longueur moyenne des larves de capelan capturées à l'aide de la pompe était constamment supérieure de 0,2 mm à celle des larves recueillies au moyen d'un filet, mais la distribution des fréquences des longueurs des larves prélevées était semblable à celle des larves se déplaçant vers la zone pélagique. Le raccordement de la prise d'eau de la pompe au cul d'un filet à plancton rigide est suggéré comme méthode permettant d'éviter les erreurs lors de l'échantillonnage d'organismes dont la densité est inférieure de 1 à 5 individus/m^3.

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The use of pumps for plankton sampling began in 1887 (Gibbons and Fraser 1937; Aron 1958). Pumps have not, however, been widely used primarily because of three perceived shortcomings: (1) pumps damage or destroy the organisms they sample, (2) volumes sampled per unit time are too small, and (3) pump systems capable of sampling sufficient volume are too cumbersome to operate, particularly from small boats (Snyder 1983).

The increasing awareness that the time and space scales involved in sampling zooplankton (Steele 1978; Mackas and Boyd 1979; Haury and Wiebe 1982; Fiedler 1983) and

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ichthyoplankton (Smith 1978; Sharp 1980; Owen 1980; Fortier and Leggett 1982, 1983; Frank and Leggett 1982; Legendre and Demers 1984) should be appropriate to the biological processes being investigated has highlighted the deficiencies of standard plankton sampling gear (reviewed in Tranter and Fraser 1968; Bowles and Merriner 1978) and standard sampling methods (Omori and Hamner 1982), especially when high-frequency sampling of discrete water masses is required. This fact, coupled with the recent advances in pump design, has resulted in renewed interest in the use of pumps for plankton sampling.

Several important advantages accrue if pump sampling can be shown to yield unbiased results. First, sampling at biologically relevant time and space scales is possible; second, sample volume can be reduced, thereby minimizing processing time and eliminating the errors frequently associated with splitting of large samples (Van Guelpen et al. 1982); third, simultaneous sampling of all size-classes down to the smallest determined by the filtering mesh size used can be achieved at the same time and place; fourth, the biasing effects of varying and different filtering efficiencies, induced by clogging and the use of multiple deployed nets at different times and/or places, are eliminated.

In this paper we briefly review the literature relating to the efficiency of large-volume pumps (here defined as pumps that deliver $>0.5\ m^3$/min) used primarily in the study of larval fishes. We also describe and evaluate a recently developed large-volume pumping system suitable for deployment from small boats (~6 m). Our criterion in designing this system was that a relatively small pumped water volume integrated over the same time and distance as a standard net tow should provide estimates of the diversity and density of plankton not significantly different from estimates derived from simultaneous towed net samples.

Efficiency of Pumps versus Nets

Five major studies using large-volume pumps have investigated the relative efficiency of pump systems and two nets (Table 1). These studies yield useful generalizations concerning pump-related damage and comparative efficiency. They also raise serious questions concerning the accuracy of the techniques used to determine volumes sampled.

All of the studies examined samples for pump-related damage (see also Lenz 1972; Icanberry and Richardson 1973; Coughlan and Fleming 1978). Only Gale and Mohr (1978) found such evidence and in this case further testing demonstrated that the incidence of damage was greatly reduced by decreasing the filtering mesh size and adjusting the length of the net so that it "ballooned" during sampling. These studies also generally found that the density of organisms sampled with pumps was equal to that sampled by towed net samplers. There were, however, important differences in the efficiency of sampling various larval fish length classes (Portner and Rohde 1977; Leithiser et al. 1979; Cada and Loar 1982). Sampling efficiency also differed between day and night (Cada and Loar 1982).

In these studies, tow net diameters, pump intake diameters, the presence or absence of intake screens or expanders, intake velocities, filtering mesh sizes, and deployment varied greatly. In only two studies was the pump intake propelled through the water, and in neither case was tow speed given (Aron 1958; Cada and Loar 1982). The remaining three studies were stationary sampling comparisons that relied on local currents to move organisms past the pump intake and to fill fixed nets. The above variability may account for some of the differences in the results obtained; however, we were unable to identify anything systematic.

Only one of the studies (Portner and Rohde 1977) employed an in-line flow meter in the pump system. All others relied on previously determined volume/time calibrations to estimate volume during timed sampling. Basic hydraulic theory dictates that two conditions *must* be met for this method to be accurate: (1) pump rpm must not change during or after calibration and (2) there must be no change in total head (static head + discharge head + velocity head + head due to friction, entrance and exit losses) during or after calibration. Hence, any alteration in pump speed, length, and/or curvature of the suction or discharge hose, static head (elevation of pump with respect to water level of source), discharge head (elevation of discharge outlet with respect to pump) will significantly alter flow rate. Accumulated volumes derived from time measurements will thus be inaccurate if any one of these criteria is not met. Few papers on plankton pumps address hydraulic theory in the design and operation of pump systems (see Quayle and Terhune 1967 for one that does), and we recommend the detailed and readable paper by Miller and Judkins (1981) to any researcher using or considering the use of pump systems.

It is important to recognize the magnitude of the errors that can occur by assuming constant flow. We calculated the effect on flow of varying either rpm or head when using the pump we describe in this study (see below). At a fixed rpm of 1250, a change in total head from 9.1 to 10.6 m (Δ14%) reduced flow rate from 0.95 to 0.63 m^3/min (Δ34%). Similarly, a reduction in rpm from 1250 to 1150 (Δ8%) at constant head reduced flow rate from 0.95 to 0.57 m^3/min (Δ40%). This clearly demonstrates that minor changes in either head or rpm can have major effects on estimates of volume sampled. As the above criteria were not explicitly met in the studies reviewed, the pumped volumes reported should be viewed with caution. This renders the net/pump efficiency comparisons questionable. Portner and Rohde (1977) suspected that their flow metering system was inaccurate primarily because it may have averaged flow over too great an area.

The volumes of water pumped in the studies reviewed ranged from 15 to 86 m^3 (Table 1). If volumes of this magnitude are required to obtain equivalent density estimates as nets then the potential usefulness of pump samplers is defeated. Such volumes take more time to collect with pumps, the resulting large samples are costly to sort, and the potential scale advantages are lost.

Large-Volume Pump System

Description of Pump System

The system we developed employs a Gorman-Rupp T3A3-B self-priming centrifugal pump with an open 22.2-cm-diameter impeller capable of handling a 6.4-cm-diameter spherical solid. The pump delivers water at up to 1.7 m^3/min (depending on total head) when driven at 2150 rpm with a single V-belt coupling to a 3-hp (minimum acceptable hp) Briggs & Stratton gasoline engine. We operated the pump at a nominal 650 rpm to prevent cavitation and to minimize velocity and pressure changes. A nominal flow rate of 0.8 m^3/min was achieved at 650 rpm when coupled to 10-m (intake) and 3-m (discharge) lengths of 7.62-cm inside diameter (ID) heavy duty wire-wrapped suction hose. Time for passage through the system,

TABLE 1. Summary of major studies designed to comparatively evaluate the sampling efficie

Reference	Pump				Tow
	Type and mesh size (μm)	Flow (m^3/min)	Suction		
			Diameter (m)	Velocity (m/s)	
Aron 1958	Centrifugal, 544 silk	1.514	0.076	5.55	0.5-m-di 476 ni
Portner and Rohde 1977	Tandem propeller, 500 nitex	8.6	0.20	4.60	0.5-m-di 500 ni
Gale and Mohr 1978	Open impeller centrifugal, 400 × 800 nitex	2.5	0.10 (0.24)[b]	5.30 (0.92)[b]	0.24 × (400 ×
Leithiser et al. 1979	Fish transfer, 335 nitex	2.1	0.15	1.92	(a) 1-m-c 335 n
					(b) as in 0.5-m 363 n
Cada and Loar 1982	Open impeller 243 nitex	1.10	0.076 (0.021)[b]	4.04 (1.1)[b]	0.5-m-di Hense 243 n

[a]Estimated from data provided in paper referenced.
[b]Measured at intake, which differs in size from suction hose.

ng efficiency of various large-volume pumps and tow nets.

Tow net and mesh size (μm)	Tow or current speed (m/s)		Volume sampled (m^3)		Gear comparison protocol
	Pump	Net	Pump	Net	
0.5-m-dia. std., 476 nitex	1.7[a]	1.7[a]	15	200	50 paired hauls "near surface" (marine)
0.5-m-dia. std., 500 nitex	Local current 0.4		86[a]	44[a]	111 paired stationary samples at 4.5, 8, and 9 m (riverine)
0.24 × 0.54-m-rect., 400 × 800 nitex	Local current "moderate–strong"		—	—	7 stationary sets of 4 pump and 8 net replicates at surface and bottom (riverine)
(a) 1-m-dia. cyl.-cone, 335 nitex	Local current (a) 0.26		(a) 62	121	(a) 10 stationary pairs at 0.5–1.5 m depth (riverine)
(b) as in (a) and 0.5-m dia. std., 363 nitex	(b) 0.30		(b) 64 64	414 105	(b) as in (a) above
0.5-m-dia. Hensen net, 243 nitex	"Slowly"	1.2–1.9	17	85–100	3 sets of 3 replicates not paired in time at 0–0.5 m depth (riverine)

TABLE 2. Quantitative aspects of sampling technique for each series of net and pump comparisons. Average values and one standard deviation (in parentheses) for each variable are given.

Year	Tow net mesh (μm)	*N*	Tow speed (m/s)	Tow time (min)	Tow distance (m)	Net volume (m^3)	Pump volume (m^3)	Pump flow (m^3/min)	Intake velocity (m/s)
1981	80	6	1.6 (0.21)	3.3 (0.14)	323 (31.8)	37.1 (3.66)	2.20 (0.048)	0.66 (0.017)	2.4 (0.06)
1982	153	4	1.1 (0.23)	2.0 (0.00)	127 (27.5)	20.4 (4.16)	1.63 (0.035)	0.82 (0.017)	3.0 (0.07)
1983	153	10	1.2 (0.15)	4.3 (0.22)	301 (36.7)	53.0 (5.83)	3.68 (0.063)	0.85 (0.039)	3.1 (0.014)

determined by fluorometric measurement of the passage of concentrated rhodamine dye, was 6.0 s. Complete flushing of the dye from the system was achieved after an additional 2.5 s.

Flow rate was continuously monitored with a Signet MK315 paddle-wheel sensor housed in the discharge hose. The sensor drove a 12-V Signet MK375 flowmeter providing both flow rate (±10 L/min) and accumulated volume (±50 L). This allowed accurate determination of volume sampled irrespective of changes in pump speed or total head during sampling. The meter was calibrated by determining the time required to pump a known volume of water while the entire system was in a fixed position.

The end of the discharge hose was fitted with a 90° PVC elbow joined to a 1-m section of PVC tubing. The end of the PVC tube was fitted with a rubber lip to prevent chafing of the filtering net. A 1.5-m-long, 80-μm plankton net fitted with an 80-μm cod-end was choked around the rubber lip during sampling. This permitted the discharge tube and net to trail freely in the water, thus minimizing abrasion (Gale and Mohr 1978) and eliminating the need for an on-board collection system (Leithiser et al. 1979).

Sampling to 16-m depth required 22 m of suction hose. We employed 3.3 and 6.6-m sections equipped with "quick-fit" couplings. The sampling intake was a 7.62-cm-ID smooth bore coupler to which we attached a 20-kg Scripps wire depressor on a 0.5-m lead. The intake was suspended from a 3.2-mm steel cable leading to a 12-V reversable boom-mounted winch. Vertical winch speed under tow was 0.12 m/s. Depth integration of a sample could be achieved by continuously varying the intake depth between predetermined limits. Diver observation of the system while underway confirmed that the intake was consistently oriented in the direction of travel and parallel to the surface.

A Data Instruments pressure transducer (model AB, 0–7.0 × 10^5 Pa) hardwired to a digital voltmeter was mounted on the intake pipe immediately behind the intake. Transducer voltage output was used to determine and regulate sampling depth (±0.3 m at 35-m maximum pressure). The total cost of the system as described was approximately $4000 Cdn. in 1981.

The pumping system and winch (total weight 300 kg) was mounted in a 4-m rubber inflatable (Dunlop), which was towed alongside a 5.6-m Boston Whaler powered with a 70-hp outboard engine. The discharge hose was mounted on the whaler and was attached with a quick-fit coupling to the pump. This configuration allowed the whaler to be easily and quickly disconnected from the inflatable. When cruising from one station to another, the suction hose was floated by raising the intake above the surface while the pump was operating. This reduced drag on the system and prevented fouling the intake when operating in very shallow water. The intake is easily towed at speeds of 1–2 m/s during sampling.

Evaluation Methods of Pump Efficiency

The sampling efficiency of the pump was evaluated by comparing the catch per unit volume of several planktonic taxa sampled simultaneously by the pump and a 0.5-m-diameter porous 2-m cylinder–cone standard net. Nets of 80- and 153-μm mesh sizes were used, and each was fitted with an 80-μm mesh window cod-end.

While the mesh sizes of nets used in our comparison were smaller than those used in other studies (see Table 1), we were concerned with evaluating the capture efficiency of small zooplankters as well as fish larvae, and the short tow duration (<5 min) ensured a constant filtration efficiency. In addition, Fortier and Leggett (1983) have shown that a fixed (current-force filtration) 80-μm, 0.5-m-diameter standard net did not differ significantly from a 151-μm mesh bongo sampler towed at 1.8 m/s in capturing small capelin (*Mallotus villosus*) and herring (*Clupea harengus*) larvae.

Water volume sampled by the nets was measured with a General Oceanics 2030 flowmeter mounted between the center and the rim of the net (Tranter and Fraser 1968). Tow speed was measured by trailing a second flowmeter abeam the boat at 0.2-m depth. Care was taken to minimize local acceleration effects. All sampling comparisons were made between 400 and 800 m seaward of the shore at Bryants Cove, Newfoundland.

Three sets of comparisons were made. In 1981 an 80-μm net was towed immediately below the surface 2 m behind the pump intake. In 1982 and 1983 a 153-μm net was towed immediately below the surface 13–15 m astern of the pump sampler. The pump intake was maintained at a depth of 0.25 m for all comparisons. Quantitative details of the sampling technique are provided in Table 2.

All nets were thoroughly rinsed after each tow and the cod-end contents were immediately preserved in 4% formalin–seawater buffered with sodium borate. Samples were completely sorted for capelin (*Mallotus villosus*) larvae, other fish larvae (86% herring, *Clupea harengus*), chaetognaths, jellyfish (mainly *Rathkea*, *Bougainvillia*, and *Aglantha* spp.), hyperiid amphipods, crab zoea and megalops, and euphausiids. Indivials of these taxa were counted and removed. The remainder of each sample was filtered through a 750-μm mesh and all fish eggs and copepods in the extract were counted. If the number of copepods or eggs in the extract was large (>400) the extract was fractionated using the HML beaker technique (Van Guelpen et

TABLE 3. Number of individuals/10 m^3 of each taxon sampled by paired pump (P) and tow net (N) in mates are not significantly different under a Wilcoxon matched-pairs signed-ranks test).

	Capelin larvae		Other larvae		Copepods >750 μm		Chaetognaths		Jellyfish	
Year	N	P	N	P	N	P	N	P	N	P
1981	2.2	0.0	—	—	1873.0	2000.0	1.9	0.0	—	—
	1.4	0.0	—	—	4002.0	4525.0	3.8	0.0	—	—
	1.9	4.6	—	—	1503.0	1747.0	1.9	0.0	—	—
	2.2	0.0	—	—	238.0	518.0	1.5	9.2	—	—
	12.3	13.8	—	—	77.0	41.0	2.2	4.6	—	—
	0.8	4.7	—	—	59.0	23.0	1.8	0.0	—	—
p	0.688		—		0.156		0.563		—	
1982	67.5	215.0	38.6	38.0	19084.0	21266.0	95.8	88.6	90.4	203.0
	320.0	285.0	104.0	48.5	28537.0	22061.0	91.2	54.5	337.0	291.0
	556.0	533.0	32.8	36.4	38264.0	37430.0	264.0	249.0	206.0	255.0
	654.0	570.0	30.9	48.5	40033.0	36364.0	199.0	315.0	370.0	333.0
p	0.875		0.875		0.375		0.875		1.00	
1983	275.0	173.7	0.2	0.0	153.0	113.0	2.4	0.0	3.9	7.9
	123.0	67.6	0.0	0.0	165.0	75.7	1.0	0.0	8.6	2.7
	53.0	25.0	0.6	0.0	237.0	169.0	1.3	0.0	14.8	16.7
	55.7	61.1	0.4	0.0	146.0	128.0	2.4	0.0	14.0	5.6
	32.5	18.9	0.0	0.0	90.3	130.0	1.8	2.7	16.9	16.2
	26.5	44.4	0.0	0.0	128.0	172.0	1.0	2.8	25.7	25.0
	38.2	51.4	0.6	0.0	142.0	281.0	2.0	0.0	22.5	21.6
	41.2	40.5	0.2	2.7	329.0	338.0	1.9	0.0	33.6	27.0
	44.1	37.8	0.2	0.0	345.0	349.0	2.7	2.7	36.3	10.8
	20.3	2.7	0.0	0.0	196.0	276.0	3.0	0.0	46.3	43.2
p	0.232		0.193		0.695		0.049		0.160	
p (total)	0.216		0.625		0.571		0.076		0.463	

net (N) in 1981 (80-μm tow net) and 1982–83 (153-μm tow net) (p = probability that density esti-

fish		Hyperiid amphipods		Crab zoea		Crab megalops		Euphausiids		Fish eggs	
	P	N	P	N	P	N	P	N	P	N	P
	—	2.2	0.0	42.1	170.0	197.0	1463.0	4.1	13.8	133.0	87.0
	—	65.5	55.8	28.7	27.9	150.0	205.0	1.4	0.0	241.0	642.0
	—	57.9	59.6	22.6	27.5	392.0	541.0	3.1	4.6	76.0	184.0
	—	141.0	91.7	37.7	55.1	569.0	1789.0	17.5	4.6	20.0	64.0
	—	51.9	142.0	34.8	55.1	445.0	1298.0	32.4	27.5	20.0	18.0
	—	28.0	32.7	14.4	60.8	127.0	808.0	11.1	9.4	15.0	23.0
—		1.00		0.031		0.031		0.688		0.219	
	203.0	—	—	5.4	24.3	—	—	153.0	215.0	253.0	75.9
	291.0	—	—	24.7	103.0	—	—	341.0	388.0	224.0	90.0
	255.0	—	—	8.9	78.8	—	—	203.0	285.0	991.0	588.0
	333.0	—	—	59.3	255.0	—	—	169.0	152.0	1672.0	1218.0
00		—		0.125		—		0.250		0.125	
	7.9	—	—	0.0	0.0	—	—	5.6	10.5	159.0	94.7
	2.7	—	—	0.0	0.0	—	—	12.6	5.4	142.0	83.8
	16.7	—	—	0.0	0.0	—	—	28.5	13.9	117.0	77.7
	5.6	—	—	0.0	0.0	—	—	26.9	0.0	92.2	97.2
	16.2	—	—	0.0	0.0	—	—	5.2	0.0	91.9	59.5
	25.0	—	—	0.0	0.0	—	—	9.2	11.1	132.0	106.0
	21.6	—	—	0.0	0.0	—	—	7.2	2.7	122.0	108.0
	27.0	—	—	0.0	2.7	—	—	17.7	5.4	124.0	103.0
	10.8	—	—	0.0	2.7	—	—	16.3	10.8	149.0	83.8
	43.2	—	—	0.0	0.0	—	—	7.1	5.4	168.0	64.9
160		—		1.00		—		0.020		0.002	
463		—		0.001		—		0.409		0.033	

al. 1982), and one fraction was counted. All counts were standardized to number per 10 m^3. Capelin larvae from the first four sample pairs collected in 1983, and all sample pairs collected in 1982, were measured for total length. Herring larvae from samples collected in 1982 were also measured for total length.

The concentration of each taxon in the sample pairs was compared using the Wilcoxon matched-pairs signed-rank test following the reasoning of Posgay and Marak (1980) (i.e. small number of samples, non-normal distributions, variances not independent of means). Differences of less than 1.0 in sample comparisons were considered as zero differences except for those samples where densities were very low. Signs were randomly assigned to zero differences and tied ranks were broken by random ordering (IMSL 1979). Significance was assessed using a two-tail, $\alpha = 0.05$ criterion. Exact probability tables were consulted (Lehmann 1975). This analysis was found to be more sensitive to detecting differences in the data set than either a Wilcoxon rank-sum test or a χ^2 test. Overall correlation between sample pairs for each taxon and across all taxa was measured after a square-root transformation on the count data. The average length of capelin larvae and the average length of herring larvae in the sample pairs were compared using a Student's *t*-test ($\alpha = 0.05$, two-tail).

Efficiency of Sampling

There were no significant differences in the abundances of six of eight taxa sampled simultaneously with the pump and the 80-μm tow net. Crab zoea and megalops were sampled more effectively with the pump (Table 3).

The pump and 153-μm tow net consistently sampled five of eight taxa without significant differences when deployed simultaneously. There were, however, differences in sampling efficiencies for chaetognaths, euphausiids, and fish eggs between years. In the 1982 comparisons (153-μm net), pump efficiency for these taxa was equivalent to net efficiency. In 1983 the net was more effective. Cunner (*Tautogolabrus adspersus*) eggs constituted ~80% of the eggs sampled. The most probable cause of the greater catchability of fish eggs in nets is the typically neustonic distribution of cunner eggs in the water column (Williams 1968). As deployed, the net sampled a vertical distance of 0.5 m centered at 0.25-m depth, while the pump intake sampled a vertical distance of 0.07 m centered at 0.25-m depth. If a greater proportion of the eggs were at or very near the surface their vulnerability to net sampling would be higher. A similar result was observed by Portner and Rohde (1977) when sampling eggs of striped bass (*Morone saxatilis*). They suggested the result might be explained by innaccurate flow measurements in either the net or pump, or differential destruction. Differential destruction of eggs between the two samplers in our study is rejected as the cause of differences because there was no difference in sampling efficiencies between gear type for small jellyfish, which are extremely delicate, and because sampling fish eggs with large-volume pumps has been shown not to affect their viability (Aron 1958; Manz 1964). In addition, none of the organisms we collected by either gear showed evidence of differential damage or destruction. The absence of difference in the number of eggs sampled by the pump and the 80-μm net is enigmatic.

The superiority of nets over the pump in the capture of chaetognaths and euphausiids in 1983 may result from differential avoidance. This interpretation is given with the caveat of the low number of organisms sampled, particularly as the differences were not apparent in the 1982 data set when natural densities were significantly greater.

TABLE 4. Correlation and regression statistics for numbers per taxon caught by net vs. pump (*n*, sample size; *r*, correlation coefficient; *a*, intercept; *b*, slope). Numbers/10 m^3 were square-root transformed. Intercepts not significantly different from 0.0 and slopes not different from 1.0 at $\alpha = 0.05$ are denoted by an asterisk.

Taxon	*n*	*r*	*a*	*b*
Capelin larvae	20	0.956	0.662*	0.973*
Other larvae	14	0.905	0.207*	1.020*
Jellyfish	14	0.957	0.836*	0.902*
Fish eggs	20	0.879	1.352*	1.008*
Copepods	20	0.996	−1.309*	1.037*
Euphausiids	20	0.960	1.490*	0.801
Crab zoea	20	0.916	0.174	0.524
Chaetognaths	20	0.968	1.039	0.850
Hyperiid amphipods	6	0.797	2.165	0.674*
Crab megalops	6	0.546	—	—
Overall	160	0.988	−0.381*	1.008*

The efficiency of the pump sampler was equal to that of the nets in sampling capelin larvae, other fish larvae, copepods, and small jellyfish. The absence of significant differences may be sample size related. Similar results were obtained when all sets of comparisons were pooled within taxa for analysis, however (Table 3).

The efficiency of the two gear types was consistent across the range of densities observed in five of the taxa considered. For capelin larvae, other fish larvae, jellyfish, fish eggs, and copepods the slopes of relationships between the transformed count data for nets and pump did not differ from 1.0 and the intercepts did not differ from 0.0 (Table 4). Regression analyses indicate that the pump was consistently more efficient in the capture of crab zoea but became progressively more efficient in the capture of euphausiids as the natural densities of these animals increased. Chaetognaths and hyperiid amphipods were captured more effectively with the net at low density (<50/10 m^3), but at higher densities the pump was superior.

The average lengths of capelin larvae captured by the pump (1982, 5.02 mm; 1983, 5.09 mm) were slightly but significantly different from the average lengths of larvae captured by the net (1982, 4.87 mm; 1983, 4.82 mm). The absence of between-year differences in average length of larvae captured by net and by pump justified pooling the data within gear type. The difference in average length of larvae sampled by net and pump was 0.2 mm. This slight length difference (4%) may be of little biological importance (approximately the growth over 1 d (Jacquaz et al. 1977; Frank and Leggett 1982)) but the frequency distributions of the larval length-classes sampled indicate a systematic bias of less than 1 mm (Fig. 1A). Proportionately more large larvae were captured by the pump than the net, while the net captured more small larvae.

We also determined the size–frequency distribution of larvae collected from the beach sediments at Bryants Cove. These collections were made during a period of actual larval emergence and on the same day in 1982 as the tow comparisons. Not unexpectedly the average length of larvae collected from the sediments (4.76 mm) was slightly but significantly smaller than postemergent larvae sampled by either the net (4.85 mm) or pump (5.06 mm) (Fig. 1A). The size–frequency distributions of

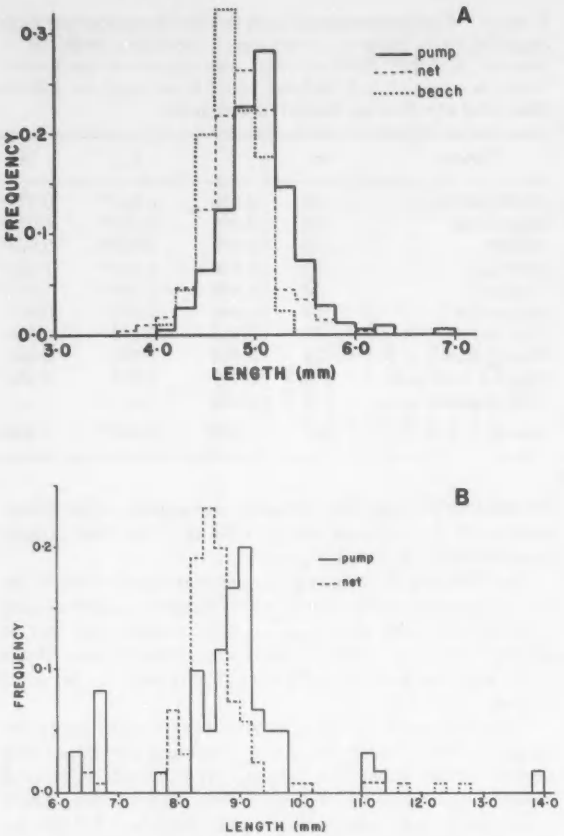

FIG. 1. Length–frequency distribution of (A) capelin larvae sampled simultaneously by pump, net (153 μm), and sampled from beach sediments and (B) herring larvae sampled simultaneously by pump and net. See text for average lengths and length differences.

beach-, net-, and pump-sampled larvae were very similar, however.

The difference in sizes of larvae sampled by pump and net is also apparent in the frequency distribution of herring larvae sampled in 1982 (Fig. 1B). In the case of herring, the average length of larvae captured by the net (8.59 mm) was smaller but not significantly different from that of larvae captured by the pump (8.78 mm). Hay (1981) reported that larvae of Pacific herring (*Clupea harengus pallasi*) experienced a shrinkage of between 13 and 17% if towed in a 350-μm net for between 1 and 3 min. He attributed the reduced length to rapid shrinkage immediately after death which occurred in the net and found that this phenomenon was independent of shrinkage due to fixation in formalin alone and that delayed fixation increased the amount of shrinkage. Qualitative observations of larvae captured by our pump showed that most, but not all, were alive and swimming immediately prior to fixing. Possibly the apparent systematic shift in length–frequency distributions results from differences in the death rate of larvae from net and pump sampling and differences in shrinkage related to death. It may also reflect microscale differences in the vertical distribution of different sizes of larvae (see foregoing discussion on vertical distribution of fish eggs).

Inferences and Application of Pump Sampling

The statistically similar density estimates obtained by simultaneous pump and net sampling for a wide variety of taxa were achieved in spite of pump volumes, which never exceeded 8% of the volumes sampled by nets. This ability to sample small volumes without compromising the accuracy of density estimates has not been addressed by previous comparative studies and has several distinct advantages: (1) a greater number of samples can be taken per unit time and per unit area, making possible greatly increased spatial and temporal resolution of plankton distributions; (2) volumes sampled are virtually error free, as net clogging, extrusion, and varying filtration efficiencies are eliminated; (3) larvae and their planktonic food can be sampled at precisely the same time and same place.

One limitation of the pump system as deployed is the time required to obtain volumes sufficient for accurate estimates of planktonic organisms occurring at densities below $1–5/m^3$. This limitation may not be important in lacustrine, estuarine, or coastal environments where densities of planktonic organisms are relatively high, but in offshore marine environments the limitation may be severe. This difficulty could be surmounted by attaching the pump intake to the cod-end of a tow net mounted on a rigid frame (see Tonolli 1951; Sasada 1982), thus eliminating net clogging and decreased filtering efficiency, which frequently occurs during prolonged net tows. Coordination between sampling depth and initiation of filtration at the discharge could serve as an "opening/closing" system. This would eliminate both the need to retrieve the net after each sample and the need to rely on mechanical opening/closing mechanisms, which frequently fail (Tuel and Knauer 1982).

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Inorganic Nitrogen Uptake in Oligotrophic Lake Taupo, New Zealand

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Priscu, J. C., and L. R. Priscu. 1984. Inorganic nitrogen uptake in oligotrophic Lake Taupo, New Zealand. Can. J. Fish. Aquat. Sci. 41: 1436–1445.

To determine nitrogen and carbon uptake in the surface waters of oligotrophic Lake Taupo, New Zealand, we used isotope tracer methods. Time-course measurements of NH_4^+ and NO_3^- uptake at near saturating concentration revealed about a fourfold greater uptake during the first hour of incubation relative to averaged 1–6 h uptake rates. This enhanced uptake was most apparent during the first 5–10 min of incubation. Uptake kinetics experiments indicated that the N assimilation rate was generally limited by low ambient concentrations of inorganic N. The half-saturation constants for N uptake were low ($\leq 10\ \mu g\ N \cdot L^{-1}$) and comparable with ambient levels of NH_4^+ and NO_3^-; NO_3^- uptake was always lower than NH_4^+ uptake and was inhibited strongly (~60%) in the presence of ~5 $\mu g\ NH_4^+$-$N \cdot L^{-1}$. Carbon to N uptake ratios averaged (±SD) 20.9 ± 11.7 (grams C to grams N) and were correlated with seston compositional ratios. Polysaccharide was the major end product of photosynthesis (as measured by ^{14}C incorporation) in measurements made at $100\ \mu E \cdot m^{-2} \cdot s^{-1}$ whereas protein was the primary end product during dark incubations. Samples enriched with NH_4^+ and incubated at $100\ \mu E \cdot m^{-2} \cdot s^{-1}$ showed a significant increase in the ^{14}C incorporated into protein, at the expense of that incorporated into polysaccharide and low molecular weight metabolites. No differences in the pathway of C flow were observed in NH_4^+-enriched, dark-incubated samples. Nitrogen uptake estimates based on the percent of ^{14}C in the protein fraction were similar to those calculated by the ^{15}N technique.

Les auteurs ont utilisé le traçage aux isotopes pour déterminer l'absorption de l'azote et du carbone dans les eaux superficielles du lac Taupo (Nouvelle-Zélande), un lac oligotrophe. Des mesures par séries chronologiques de l'absorption du NH_4^+ et du NO_3^- à des concentrations proches de la saturation ont révélé une absorption quadruplée pendant la première heure d'incubation par rapport aux taux d'absorption moyens après 1 à 6 h. Cette absorption accrue était plus apparente pendant les premières 5 à 10 min d'incubation. Des expériences sur la cinétique de l'absorption ont montré que le taux d'assimilation du N était généralement limité par de faibles concentrations ambiantes de N inorganique. Les valeurs des constantes de demi-saturation pour l'absorption du N étaient peu élevées ($\leq 10\ \mu g\ N \cdot L^{-1}$) et comparables aux concentrations ambiantes de NH_4^+ et de NO_3^- ; l'absorption de ce dernier était toujours plus faible que celle du NH_4^+ et était fortement inhibée (~60 %) par la présence d'environ 5 $\mu g\ N$-$NH_4^+ \cdot L^{-1}$. Le rapport d'absorption entre le carbone et le N (grammes C à grammes N) s'élevait en moyenne à 20,9 ± 11.7 (E.-T.) et était relié au rapport entre les composantes du seston. Les polysaccharides représentaient le principal produit final de la photosynthèse dosage du ^{14}C incorporé et mesures effectuées à $100\ \mu E \cdot m^{-2} \cdot s^{-1}$ tandis que les protéines étaient le principal produit final pendant l'incubation à l'obscurité. Les échantillons enrichis de NH_4^+ et incubés à $100\ \mu E \cdot m^{-2} \cdot s^{-1}$ présentaient une augmentation importante du ^{14}C incorporé dans les protéines aux dépens de celui incorporé dans les polysaccharides et les métabolites de faible poids moléculaire. Aucune différence de la structure du transport C nà été observée dans les échantillons enrichis de NH_4^+ et incubés à l'obscurité. Les estimations de l'absorption de N basées sur le pourcentage de ^{14}C dans la fraction protéique étaient semblables à celles calculées à l'aide de la technique au ^{15}N.

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The growth of phytoplankton is often regulated by the supply of dissolved inorganic nitrogen (DIN). Consequently, an understanding and quantification of DIN utilization by phytoplankton is essential to explain the effect of N supply on primary production. To date, most of our knowledge about DIN utilization by phytoplankton has been derived from studies on naturally occurring or laboratory grown marine organisms (reviewed by Collos and Slawyk 1980; McCarthy 1980, 1981). These studies have ranged from measurements of the distribution of DIN in natural water (Vaccaro and Ryther 1960; Vaccaro 1965) to detailed studies of the physiological state of algae under a variety of nutrient conditions (e.g. McCarthy and Eppley 1972; Dortch 1982; Horrigan and McCarthy 1982).

Although N has been implicated as a factor controlling the growth of phytoplankton in a number of freshwater systems (e.g. Forsberg 1977; Murphy 1980; Axler et al. 1982),

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relatively little information exists on the rates of DIN turnover, the relative nutritional importance of the various DIN forms, and the effect of substrate concentration on the rate of uptake. Moreover, to our knowledge, no information exists regarding the ability for enhanced uptake rates over short time periods following pulses of DIN in natural populations of freshwater phytoplankton, although such an ability has been invoked for the euphotic zone of several lakes (Liao and Lean 1978; McCarthy et al. 1982; Axler et al. 1981, 1982; Priscu and Goldman 1983a). An increased knowledge of the dynamics of DIN in freshwater ecosystems will allow broader generalizations to be drawn regarding the nitrogenous nutrition of phytoplankton in natural waters.

Lake Taupo, the largest lake in Australasia, is an oligotrophic lake of 612 km^2 and 97 m average depth, situated on the Central Volcanic Plateau of the North Island, New Zealand. White and Downes (1976) estimated the overall ratio of DIN to dissolved reactive phosphorus (DRP) of the inputs to the lake to be about 5.7 by weight, and this led them to suggest that N may be an important growth-limiting nutrient. Subsequent work using several bioassay techniques has shown that DIN can limit phytoplankton growth in this lake, particularly during winter mixing (July–December) (White and Payne 1977; Vincent 1981, 1983). Our objectives were to utilize the isotope ^{15}N to perform more direct bioassays for N limitation in Lake Taupo and to examine in detail the uptake of NH_4^+ and NO_3^- as a function of substrate concentrations and time. By comparing the rates of inorganic N uptake to photosynthetic rates and the major end products of photosynthesis, we would gain insight into how N availability influences cell chemistry and metabolism.

Materials and Methods

All samples were collected with a 5-L Van-Dorn bottle between 6 July and 26 August 1982 near midmorning from Tapuaeharuru Bay, a large bay in the northeastern portion of the lake, at a depth of 5–10 m unless otherwise noted.

N Experiments

Nitrogen uptake kinetics were determined using the stable isotope ^{15}N as a tracer. Lake water, prefiltered through 200-μm netting to remove most of the larger zooplankton, was added to 500-mL glass-stoppered bottles and inoculated with $(^{15}NH_4)_2SO_4$ (99 atom-% ^{15}N) or $K^{15}NO_3$ (95.5 atom-% ^{15}N) to final concentrations ranging from 1 to 110 μg N·L^{-1}. After 3–5 h of laboratory incubations at the temperature and photon flux density (100 μE·m^{-2}·s^{-1}; cool-white fluorescent) of collection, the samples were filtered onto precombusted glass-fiber filters (Whatman GF/F).

Ambient rates of NH_4^+ and NO_3^- uptake were calculated by assuming that the rate of uptake was related to the ambient NH_4^+ and NO_3^- concentrations according to the Michaelis–Menten equation

$$(1)\quad V = V_{max}\frac{S}{K_s + S}$$

where V is the specific rate of uptake, S is the substrate concentration (^{14}N + ^{15}N), V_{max} is the rate of uptake at saturating levels of S, and K_s is the half-saturation constant (at which $V = V_{max}/2$). The kinetic constants K_s and V_{max} were calculated by the Hanes–Woolf linear transformation of the Michaelis–Menten equation (Segel 1976)

$$(2)\quad \frac{S}{V} = \frac{1}{V_{max}}S + \frac{K_s}{V_{max}}.$$

Time-course uptake of NH_4^+ and NO_3^- was determined on prescreened (200 μm) water samples incubated in the laboratory as described above. Samples were enriched with ^{15}N to a final concentration of 2 and 100 μg N·L^{-1} plus the ambient ^{14}N; the reactions were terminated by filtration at intervals between 5 min and 7 h. Each filtration (Whatman GF/F) took approximately 40 s, a time period accounted for in our rate calculations. Controls to check for ^{15}N adsorption to the glass-fiber filters consisted of filters that had 250 mL of ^{15}N (NO_3^- and NH_4^+) enriched (~5 atom-%) distilled water passed through them followed by an appropriate volume of unenriched lake water to bring the particulate nitrogen (PN) concentration up to a level required for ^{15}N determination. These controls failed to show measurable ^{15}N enrichment.

The influence of NH_4^+ concentration on NO_3^- uptake was evaluated by measuring NO_3^- uptake at NH_4^+ enrichments from 0 to 100 μg NH_4^+-N·L^{-1}. All samples were allowed to preincubate for 1 h with NH_4^+ prior to the addition of $^{15}NO_3^-$ to a final concentration of 100 μg $^{15}NO_3^-$-N·L^{-1}. A separate set of samples was treated with L-methionine-DL-sulphoximine (MSX) (100 μmol·L^{-1}) and allowed to preincubate for 45 min prior to the NH_4^+ and $^{15}NO_3^-$ enrichments. This compound inhibits glutamine synthetase (GS) activity and thus glutamine formation (Ronzio et al. 1969), thereby allowing examination of some of the physiological mechanisms by which NH_4^+ inhibits NO_3^- uptake. All samples were incubated with the isotope for 6 h under artificial lighting (100 μE·m^{-2}·s^{-1}; cool-white fluorescent) at the temperature of collection.

An experiment designed to measure dark NH_4^+ uptake in ambient and NH_4^+ enriched (N-sufficient) samples was set up essentially as outlined by Axler and Goldman (1981). Prescreened (200 μm) lake water was added to six flasks, three of which were enriched to 60 μg NH_4^+-N·L^{-1}. All samples were then incubated at 100 μE·m^{-2}·s^{-1} (cool-white fluorescent) at the temperature of sample collection for 22 h. The NH_4^+ and PN concentration of the samples was then determined and appropriate amounts of $^{15}NH_4^+$ were added to each flask to bring the NH_4^+ concentration (^{14}N + ^{15}N) to 120 μg NH_4^+-N·L^{-1}. This level of NH_4^+ addition was chosen so that ^{15}N uptake rates would be substrate saturated and relatively insensitive to differences in NH_4^+ concentration that might occur during the $^{15}NH_4^+$ incubations. No significant changes in the PN concentrations of the samples occurred during this incubation. The samples were then incubated for an additional 6 h in the dark with the isotope to determine uptake rates.

^{15}N Determination

After drying (65°C for 24 h), the ^{15}N samples were prepared for emission spectrometry by the modified Dumas method according to Kanazawa and Yoneyama (1976). Metallic Cu was added to the cupric oxide (4:3 CuO:Cu by weight) to ensure complete reduction of nitrogen oxides during the combustion. A Packard model NOI-5 emission spectrometer was used to measure the fraction of ^{15}N in the assay gas. Optimal emission was obtained with a sample size of 3–5 μg of N. The instrument was standardized with known enrichments of ^{15}N-labelled urea determined by mass spectrometry as outlined by Blackburn (1979) and Keeney and Tedesco (1973). The average coefficient

of variation for seven standards (five replicates per standard) between 1.36 and 9.79 ^{15}N atom-% was 2.5%; the average CV of five standards each at 0.366 and 0.713 ^{15}N atom-% was 12.5%. The fraction of ^{15}N in the assay gas was determined according to Keeney and Tedesco (1973) and the rates of uptake (ρ) according to Neess et al. (1962) and Dugdale and Goering (1967). Specific rates of nitrogen uptake (V) were obtained by dividing the volumetric uptake (ρ) by the PN concentration at the beginning of the experiment. One should note that specific uptake rates calculated in this manner can be misleading, particularly when referred to as a growth coefficient, since an unknown and perhaps significant portion of the PN (and particulate carbon (PC)) may be nonliving. The uptake rates may also be underestimates because corrections were not made for isotopic dilution by regenerated ^{14}N (Glibert et al. 1982).

^{14}C Methodology

Phytoplankton photosynthesis was determined at the same time as DIN uptake on 130-mL samples (two light, two dark), each inoculated with 148 kBq $Na_2{}^{14}CO_3$ and incubated under conditions described for N uptake measurements. The incubations were terminated after 3 h by filtering 20–50 mL onto glass-fiber filters (Whatman GF/F). The remainder of the sample from each of the bottles was filtered through a second filter (Whatman GF/F), which was stored frozen in 1.2 mL of distilled water. These filters were subsequently analyzed by the method outlined by Priscu and Priscu (1984) to determine the major end products of photosynthesis (EPPS). This procedure allows the assimilated ^{14}C to be apportioned into chloroform soluble (lipid), methanol–water soluble (low molecular weight metabolites), hot trichloroacetic acid (TCA) soluble (polysaccharide), and TCA insoluble (protein) fractions.

A separate experiment examined the patterns of ^{14}C incorporation into the EPPS at both ambient and enriched (+100 μg $NH_4{}^+$-N·L^{-1}) N concentrations. These samples were incubated in the laboratory for 3–4 h at 100 μE·m^{-2}·s^{-1} and the ambient lake temperature, after which they were filtered and fractionated.

The photosynthetic rates of the >5 μm and 0.2–5 μm algal fractions of ambient and $NH_4{}^+$-enriched (+100 μg $NH_4{}^+$-N·L^{-1}) samples were obtained by differential filtration. The treatments (in triplicate) were incubated with ^{14}C and $NH_4{}^+$ (enriched samples only) for 20 h whereupon 10 mL was filtered from each flask through a 5-μm Nuclepore filter followed by a 10-mL distilled water rinse. The filtrate was then refiltered through a 0.2-μm Nuclepore filter and rinsed. The ^{14}C activity on the filters was used to calculate photosynthetic rates. The filtrate from the 0.2-μm filtration, refiltered through separate 5- and 0.2-μm filters, served as abiotic blanks.

Enzyme Assays

The organisms in 2–4 L of 200-μm prescreened lake water were concentrated onto glass-fiber filters (Whatman GF/F) under low vacuum (<100 kPa). Visible zooplankton that appeared on the filters were removed manually with forceps. Nitrate reductase (NR) and glutamic acid dehydrogenase (GDH) were extracted in 3–5 mL of homogenization buffer with the following composition: 75 μmol $MgSO_4$·L^{-1}, 0.2% (v/v) Triton X-100, 0.15% (w/v) PVP, 50 mmol sodium phosphate buffer·L^{-1}, pH 8.0. The filter and cells were homogenized in this solution for 2 min with a Teflon tissue grinder at 0°C prior to centrifugation at 4000 × g for 10 min at 0°C. NR activity was determined by the method of Lowe and Evans (1964) except that methyl viologen was used as the electron donor (Jones 1979). Samples were incubated for 1 h at 35°C. GDH activity was determined fluorometrically by measuring the rate of NADPH oxidation at 35°C over 30 min as described by Lowry and Passonneau (1972) and Ahmed et al. (1977). GS was extracted by homogenization (2 min at 0°C) in 3–5 mL of imidazole buffer containing 0.2% Triton X-100, pH = 7.0, and assayed with the γ-glutamyl transfer reaction (Shapiro and Stadtman 1970); incubation was for 45 min at 35°C. The reactions of all the enzymes were found to be linear for at least 1 h at the biomass levels used. Specific enzyme activity (per hour) was obtained by normalizing enzyme activities to PN concentrations.

Particulate Analysis

Particulate phosphorus (PP) and PN were determined by Kjeldahl digestion of filtered samples (precombusted Whatman GF/F) with subsequent analysis of DRP and $NH_4{}^+$ (Priscu and Priscu 1984). PC was determined with a LEECO carbon analyzer standardized with glucose. Pheophytin-corrected chlorophyll *a* was determined by fluorometric analysis of acetone extracts before and after acidification (Strickland and Parsons 1968). Phytoplankton enumeration was done on 50-mL samples that had been fixed with acid–Lugol's solution and settled for at least 2 d using the inverted microscope technique (Lund et al. 1957).

Physical and Chemical Methods

Samples for nutrient analysis were filtered through acid-washed glass-fiber filters (Whatman GF/C). $NO_3{}^-$ was analyzed by the automated hydrazine reduction method of Downes (1978) and $NH_4{}^+$ by the automated method of Crooke and Simpson (1971) or manually on 10-mL samples by the method of Solórzano (1969). Photon flux density was measured with a Li-Cor quantum sensor and temperature with a YSI 54A thermistor.

Results

General Characteristics

Water temperature at 10 m averaged 11.3°C (Table 1) and was uniform with depth (J. C. Priscu, unpubl. data), indicating that the water in Tapuaeharuru Bay was mixed completely to the bottom (~40 m) during the sampling period. The mean $NO_3{}^-$ concentration was 0.8 μg $NO_3{}^-$-N·L^{-1} (range 0.3–1.3) whereas the mean $NH_4{}^+$ concentration measured by the wet chemical methods was 2.9 μg $NH_4{}^+$-N·L^{-1} (range 1.5–3.4). Because these $NH_4{}^+$ concentrations seemed unusually high relative to $NO_3{}^-$, and moreover because they led to uptake kinetics data that did not follow expected saturation kinetics, we used the $NH_4{}^+$ uptake kinetics data to estimate what appear to be more realistic concentrations (mean corrected ambient concentration = 0.5 μg $NH_4{}^+$-N·L^{-1}, range = 0.4–0.6). Chlorophyll *a*, PC, PN, and PP concentrations were correlated, attaining their highest levels on 3 August (Table 1). The phytoplankton community during the study was composed entirely of diatom and green algae (Table 2).

Time-Course Uptake of $NH_4{}^+$ and $NO_3{}^-$

The specific $NH_4{}^+$ uptake rate V (per hour) calculated at two levels of enrichment was enhanced approximately fourfold

TABLE 1. General limnological characteristics in Lake Taupo during the study period. Samples (mean ± SD) were collected at 10 m during winter mixing. PC:PN and PN:PP ratios are by weight. Parenthetic values of NH_4^+ were measured by wet chemistry; italicized values are from NH^+ uptake kinetics experiments (see text for details).

Date	Temp. (°C)	$\mu g \cdot L^{-1}$							
		NO_3^-–N	NH_4^+–N	Chl *a*	PC	PN	PP	PC:PN	PN:PP
7 July 1982	—	0.9	*0.6* (3.4)	0.76±0.09	269.0±54.3	27.8±2.3	1.8±0.2	9.7	15.4
3 Aug. 1982	11.0	0.3	*0.5* (1.8)	1.52±0.15	576.0±19.6	36.6±1.8	3.4±0.2	15.7	10.8
26 Aug. 1982	11.5	1.3	*0.4* (3.4)	0.72±0.04	196.6±7.4	19.9±2.3	2.0±0.2	9.9	10.0

TABLE 2. Phytoplankton abundance in water samples collected at 10 m from Lake Taupo.

Organism	$cells \cdot mL^{-1}$		
	6 July	22 July	26 Aug.
Bacillariophyceae			
Cyclotella	200	0	518
Melosira	570	388	583
Synedra	0	9	10
Asterionella	15	19	19
Navicula	26	9	0
Epithemia	0	9	0
Chlorophyceae			
Ankistrodesmus	355	157	65
Flagellates			
>3 μm	910[a]	425	194
<3 μm		314	120
Green ovoid cells			
~3 μm	503	1369	1701

[a]These organisms were not separated into size-classes.

during the first hour of incubation relative to the average 1–6 h value of *V* (Fig. 1A, 1B). Based on the average rates of uptake over the first hour of incubation, 1.5 and 52.2% of the available NH_4^+ was removed from the high (100 μg NH_4^+-N·L^{-1}) and low (2 μg NH_4^+-N·L^{-1}) enriched samples, respectively. Because of the low K_s value, both of these enrichments were greater than 95% of saturation. After 1–2 h of incubation, the uptake rates were virtually constant to 6 h. Therefore, even though the organisms could continue to remove NH_4^+ from the media, as indicated by the continuous increases in ^{15}N atom-% excess, the enhanced rates were not sustained.

Time-course uptake of NO_3^- also showed an enhancement during the first hour of incubation (Fig. 1C, 1D). The average uptake during this time was about 10-fold higher than rates obtained after 1 h at both 100 and 2 μg NO_3^--N·L^{-1} enrichments (ambient NO_3^- = 1.3 μg NO_3^--N·L^{-1}). However, the time-course changes in ^{15}N atom-% excess were quite different from those obtained with NH_4^+. Whereas the increase in $^{15}NH_4^+$ atom-% excess was relatively gradual with time and could be easily extrapolated to zero-time, the $^{15}NO_3^-$ atom-% excess increased abruptly sometime during the first 5 min of incubation, making an extrapolation to zero-time difficult. We feel that zero-time should be considered a valid datum point because, by expressing the data as atom-% excess, we have taken the ^{15}N enrichment at this point into account. After this initial increase, the atom-% excess in the 100 μg NO_3^--N·L^{-1} enrichment changed little up to 5.5 h, after which a single measurement at 6.4 h showed about 1.5-fold increase. A similar trend in atom-% excess was observed in the 2 μg NO_3^--N·L^{-1} enrichment experiment. The enrichments of 100 and 2 μg NO_3^--N·L^{-1} were about 97 and 55% of V_{max}.

To determine if enhanced uptake capacity was related to the nutritional state of the phytoplankton, we measured the dark uptake rate of NH_4^+ in enriched (preincubated with 60 μg NH_4^+-N·L^{-1} for 22 h) and unenriched lake water as proposed by Fitzgerald (1969). The NH_4^+ uptake rate of the unenriched samples (0.019 ± 0.004·h^{-1}) was significantly greater (*t*-test; $p < 0.05$) than that of the enriched (N-sufficient) samples (0.010 ± 0.003·h^{-1}), indicating that apparently N-deficient organisms can lose their overall capacity for enhanced uptake when supplied with N as NH_4^+.

Uptake Kinetics

NO_3^- and NH_4^+ uptake kinetics experiments were done with 10-m water samples on 7 July and 26 August (Fig. 2, 3). NO_3^- uptake followed saturation kinetics allowing the computation of K_s and V_{max}. The K_s values for NO_3^- were 10.9 and 2.7 μg NO_3^--N·L^{-1} for the above dates, respectively, both of which were greater than the ambient NO_3^- concentrations at the time of the experiments. The V_{max} for NO_3^- on 7 July (0.005·h^{-1}) was identical to that calculated on 26 August.

In contrast with the uptake kinetics of NO_3^-, NH_4^+ uptake was not directly consistent with Michaelis–Menten kinetics. In both experiments the velocity of uptake decreased as the NH_4^+ concentration increased (Fig. 3). As discussed by Murphy (1980), this irregularity can be eliminated if it is assumed that the chemical analysis overestimates ambient NH_4^+ concentration. The term "ambient" NH_4^+ is used to distinguish the natural concentration of NH_4^+ in the sample from that which is added to an incubation or that which is due to remineralization of organic matter. Subsequent work by M. Timperley in our laboratory has confirmed that a percentage (~3%) of the ambient dissolved organic nitrogen (DON) can be hydrolyzed and appear as NH_4^+ in the analytical methods we employed.

The shape of the uptake kinetics curve may also be influenced by variations in the kinetics constants of different size fractions of organisms (Sherr et al. 1982; Lean and White 1983). We obtained indirect evidence that the organisms in Lake Taupo in the size range between 0.2 and 5 μm can respond to NH_4^+ addition differently than those larger than 5 μm (Table 3; see also Table 2). Measurements of ^{14}C uptake in control and enriched (+100 μg NH_4^+-N·L^{-1}) lake water revealed that the photosynthesis of organisms between 0.2 and 5 μm was stimulated significantly by the NH_4^+ enrichment ($p < 0.05$) whereas those larger than 5 μm showed no statistically significant response ($p > 0.50$). Interestingly, the difference observed for the 0.2–5 μm fraction was masked ($p < 0.5$) when the community was viewed as a whole (>0.2 μm) even though the ^{14}C uptake by small organisms accounted for more than 50% of the total C uptake.

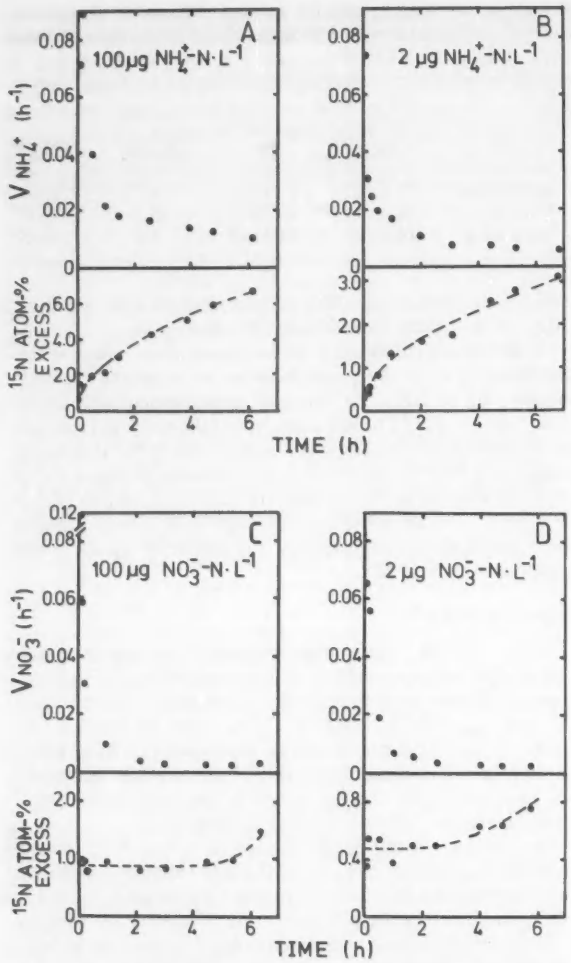

FIG. 1. Time-course of NH_4^+ (A and B) and NO_3^- (C and D) uptake at 100 and 2 µg $N \cdot L^{-1}$ enrichments. Top panels are time-integrated specific uptake rate V (h^{-1}) and bottom panels are ^{15}N atom-% excess in seston. Ambient NH_4^+ (kinetically determined) and NO_3^- concentrations were 0.4 and 1.3 µg $N \cdot L^{-1}$, respectively. The N concentration ($^{14}N + {}^{15}N$) at the beginning of each experiment was at V_{max} for experiments shown in Fig. 1A, 1B, and 1C and 55% of V_{max} in Fig. 1D. The curves were drawn by inspection to indicate trends only.

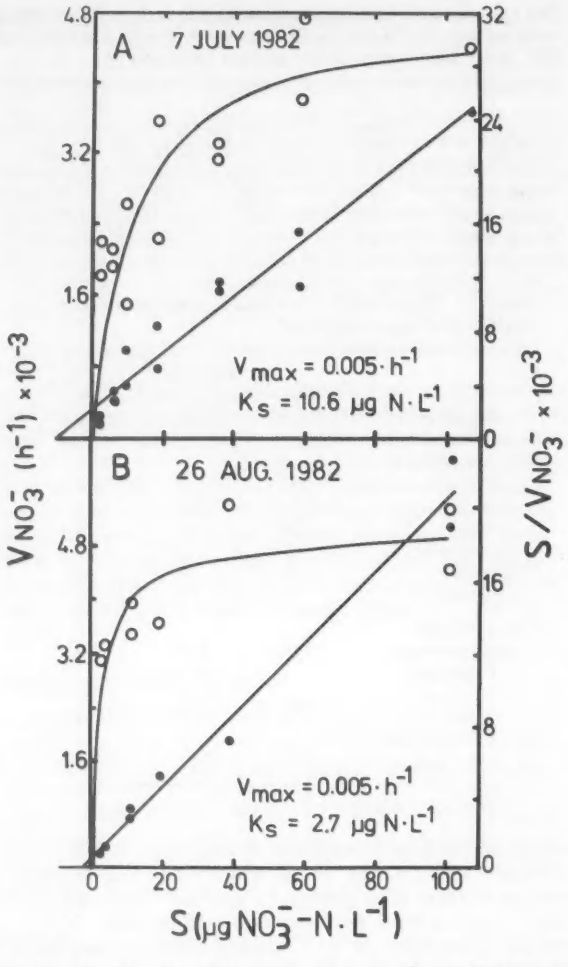

FIG. 2. Specific rate of NO_3^- uptake $V_{NO_3^-}$ (h^{-1}) as a function of total NO_3^- ($^{14}N + {}^{15}N$) available (○) on (A) 7 July and (B) 26 August 1982. Also shown is the S/V vs. S transform of the data (●) used to calculate the Michaelis–Menten kinetic parameters V_{max} (h^{-1}) and K_s (µg NO_3^--$N \cdot L^{-1}$). Note that the values on the y-axis must be multiplied by 10^{-3}.

Assuming that our data should conform to Michaelis–Menten theory and that our ambient NH_4^+ concentrations were overestimated, we used the Michaelis–Menten equation to calculate a theoretical upper limit of NH_4^+ concentration in Lake Taupo. Based on these relationships, we calculated concentrations of 0.6 and 0.4 µg NH_4^+-$N \cdot L^{-1}$ as the levels where K_s just (± 0.1 µg $N \cdot L^{-1}$) became positive in the 7 July and 26 August experiments, respectively. All of the NH_4^+ uptake data we present are based on these kinetic determinations of ambient NH_4^+. Thus, the K_s values for NH_4^+ uptake were 0.05 and 0.09 µg NH_4^+-$N \cdot L^{-1}$ on 7 July and 26 August, respectively, which are several orders of magnitude lower than those for NO_3^-. The V_{max} values for NH_4^+ on these dates were 0.009 and 0.012·h^{-1}, respectively, which are about twice those for NO_3^-.

Rates of C and N uptake at ambient and saturating substrate levels are compared in Table 4. Carbon uptake (ρC) and $NH_4^+ + NO_3^-$ uptake (ρN) were positively correlated ($r^2 = 0.99$). The ratios of PC:PN (see Table 1) were also correlated ($r^2 = 0.98$) with ρC:ρN, implying that, even though a large proportion of the PC and PN may be nonalgal, the ratio of these elements can be influenced by the relative proportions of dissolved inorganic C and N utilized by the phytoplankton. Dark uptake of NH_4^+ and NO_3^- averaged 51 and 77%, respectively, of that in the light during 3–5 h incubations.

Because ρNO_3^- was always less than 20% of ρN ($\bar{X} = 11\%$), we investigated some of the factors influencing NO_3^- uptake. Nitrate uptake (at V_{max}), measured in the presence of variable amounts of NH_4^+, indicated that only about a 5 µg NH_4^+-$N \cdot L^{-1}$ enrichment was required to inhibit NO_3^- uptake by 60% of that in unenriched samples (Fig. 4). Unfortunately, we could not determine the amount of inhibition caused by the ambient NH_4^+

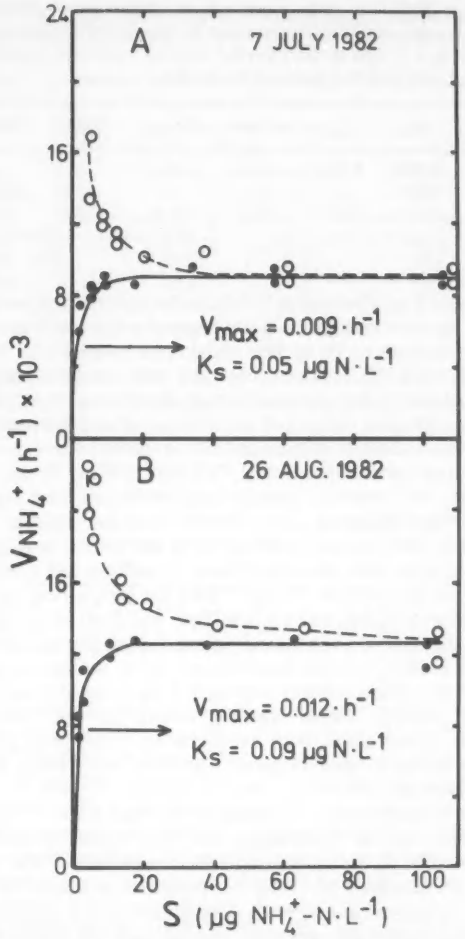

FIG. 3. Specific rate of NH_4^+ uptake $V_{NH_4^+}$ (h^{-1}) at different ambient NH_4^+ concentrations as a function of total NH_4^+ ($^{14}N + {}^{15}N$) available. ●, assuming ambient NH_4^+ levels of (A) 0.6 and (B) 0.4 µg N·L^{-1}; ○, using the chemically determined ambient concentration of 3.4 µg N·L^{-1}. The experiments were done on (A) 7 July and (B) 26 August 1982. The Michaelis–Menten constants V_{max} (h^{-1}) and K_s (µg NH_4^+-N·L^{-1}) were calculated from the highest NH_4^+ concentration (0.6 and 0.4 µg N·L^{-1} in Fig. 3A and 3B, respectively) that just (±0.1 µg N·L^{-1}) yielded a positive K_s value, as determined from an S/V vs. S transformation (not shown). See text for details. Note that the values on the y-axis must be multiplied by 10^{-3}.

concentration, which could be considerable. This last contention is supported by the low specific rates of NR activity ($\bar{X}$ ± SD) measured in unenriched lake water samples (0.001 ± 0.001·h^{-1}), which, incidentally, are similar to the specific rates of NO_3^- uptake at ambient NO_3^- concentrations (0.002·h^{-1}) on both 3 and 26 August).

Nitrate uptake in samples incubated at 100 µg NH_4^+-N·L^{-1} after pretreatment with 100 µmol MSX·L^{-1}, an inhibitor of GS activity, did not differ significantly from untreated samples. We suggest that either GS was not a major enzyme of N assimilation during the experiment or that glutamine, the end product of the GS-catalyzed reaction, does not suppress NO_3^- uptake through feedback inhibition, as has been proposed previously (Rigano et al. 1982; Cullimore and Sims 1981). Measurements of GS and GDH (the latter catalyzes an alternate pathway of N assimilation (Miflin and Lea 1977)) revealed that potential GS activity (0.215 ± 0.008·h^{-1}) was threefold greater than potential GDH activity (0.070 ± 0.019·h^{-1}); this leads us to conclude that under our experimental conditions, glutamine per se does not inhibit NO_3^- uptake.

TABLE 3. Influence of N enrichment on ^{14}C uptake for different size-classes of surface organisms in Lake Taupo, 22 July 1982. (+N = 100 µg NH_4^+-N·L^{-1}). Statistical significance determined by a t-test (n.s. = $p > 0.05$).

Size-class	Mean dpm ± SD	
	Control	+N
>5 µm	3780±243	3646±596 (n.s.)
0.2–5 µm	4251± 67	4485±103 ($p < 0.05$)
Community response (>0.2 µm)	8031±246	8131±527 (n.s.)

End Products of Photosynthesis

The photosynthetic pattern of ^{14}C incorporation into lipid, polysaccharide, protein, and low molecular weight metabolites, which correspond to the ρC measurements in Table 4, is shown in Table 5. A majority of the ^{14}C flowed into the polysaccharide fraction ($\bar{X}$ = 44.0%) in experiments conducted at 100 µE·m^{-2}·s^{-1}. In the same experiments, the average ^{14}C incorporated into protein (22.0%) and low molecular weight metabolites (19.6%) was similar, while only 14.4% was observed as the average in the lipid fraction. Experiments conducted on darkened samples showed a general increase in the protein fraction ($\bar{X}$ = 37.7%), primarily at the expense of the polysaccharide ($\bar{X}$ = 32.3%) and low molecular weight metabolite ($\bar{X}$ = 12.5%) fractions The lipid fraction also increased slightly ($\bar{X}$ = 17.5%) under dark conditions.

The protein fraction increased significantly ($p < 0.01$) in samples enriched with 100 µg NH_4^+-N·L^{-1} and incubated at 100 µE·m^{-2}·s^{-1} (Table 6). At the same time, a significant ($p < 0.01$) decrease in ^{14}C incorporation was noted in the law molecular weight metabolite fraction. The ^{14}C incorporated into the lipid and polysaccharide fractions was not altered significantly ($p < 0.1$) by the NH_4^+ enrichment. Enrichment by NH_4^+ of the darkened samples increased ^{14}C flow into the polysaccharide fraction (+5.8%) and decreased that into the low molecular weight metabolite fraction (−7%); however, these changes as well as those observed in the lipid (+1.9%) and protein (−0.7%) fractions were not statistically significant ($p < 0.1$). Importantly, although we noted considerable changes in the intracellular pathway of C flow, the total ^{14}C uptake by the phytoplankton was not altered significantly ($p < 0.5$) by the addition of NH_4^+ in either the light or dark treatments.

Assuming that the fraction of protein C in phytoplankton is 2.8 N:C, where N:C is the N to C ratio by weight in the phytoplankton (Mayzaud and Martin 1975; DiTullio and Laws 1983), the N uptake based on the percent ^{14}C incorporated into protein was 0.358, 0.613, and 0.233 µg N·L^{-1}·h^{-1} for 7 July, 3 August, and 28 August, respectively. These rates, although slightly higher, are in good agreement with ρN obtained by the ^{15}N method (see Table 4).

TABLE 4. Uptake rates for inorganic C (ρC) (μg C·L^{-1}·h^{-1}) and N (ρN = ρNO$_3^-$ + ρNH$_4^+$ and ρ_{max} = ρNO$_3^-{}_{max}$ + ρNH$_4^+{}_{max}$) (μg N·L^{-1}·h^{-1}) in 10-m water samples. Also included are V_C, V_N, V_{max} = specific rates (h^{-1}) normalized to PC and PN concentrations, respectively; C:N uptake ratios, ρC:ρN and ρC:ρ_{max} (g C:g N); and the ratios of dark to light uptake for NH$_4^+$ (DNH$_4^+$) and NO$_3^-$ (DNO$_3^-$). NH$_4^+$ uptake rates were calculated using kinetically determined concentrations of ambient NH$_4^+$ (see text for details).

Date	ρC	ρNH$_4^+$	ρNO$_3^-$	ρN	ρ_{max}	V_C	V_N	V_{max}	ρC:ρN	ρC:ρ_{max}	DNH$_4^+$	DNO$_3^-$
7 July 1982	2.78	0.227	0.0004	0.227	0.379	0.010	0.008	0.014	12.3	7.3	—	—
3 Aug. 1982	14.01	0.337	0.074	0.411	—	0.024	0.011	—	34.2	—	0.56	0.90
26 Aug. 1982	3.70	0.198	0.032	0.230	0.341	0.019	0.012	0.017	16.1	10.9	0.46	0.64

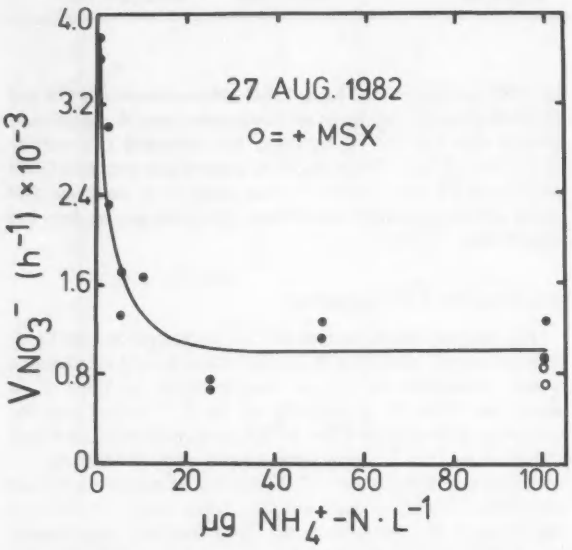

FIG. 4. Specific rate of NO$_3^-$ uptake $V_{NO_3^-}$ (h^{-1}) as a function of NH$_4^+$ concentration (μg NH$_4^+$-N·L^{-1}) on 26 August 1982. The NO$_3^-$ concentration (^{14}N + ^{15}N) was at saturation (101.3 μg NO$_3^-$-N·L^{-1}); the kinetically determined ambient NH$_4^+$ concentration was 0.4 μg NH$_4^+$-N·L^{-1}. O, samples that were preincubated with 100 μmol MSX·L prior to the NH$_4^+$ and the NO$_3^-$ additions (see text for details). The curve was drawn by inspection. Note that the values on the *y*-axis must be multiplied by 10^{-3}.

Discussion

Time-Course Uptake of N

The enhanced NH$_4^+$ uptake during the first minutes of incubation supports the laboratory and field observations on marine organisms (e.g. Glibert and Goldman 1981; Dortch et al. 1982; Wheeler et al. 1982). These results have been interpreted as a physiological adaptation of marine phytoplankton to N limitation (Dugdale 1977; Dortch et al. 1982) and as a more specific adaptation to extensive spatial and temporal variability in NH$_4^+$ supply (McCarthy and Goldman 1979). Glibert and Goldman (1981) and Wheeler et al. (1982) have further shown that the degree of nonlinearity in NH$_4^+$ uptake (i.e. the degree of enhancement with time) by natural marine phytoplankton increases as the ambient NH$_4^+$ concentration decreases. It appears that the phytoplankton in Lake Taupo can respond to a N-deficient environment in much the same manner as N-deficient marine populations. We suggest that the phytoplankton in Lake Taupo were N-deficient because of the enhanced V_{max} observed in N-deficient samples during our dark NH$_4^+$ uptake experiments. They respond to this condition with a short-term elevation of their initial uptake capacity for N.

The rapid NH$_4^+$ uptake observed over the first 5 min of incubation may have resulted from physical transport processes such as diffusion across cell membranes, adsorption onto cell surfaces, or both (Goldman and Glibert 1982). However, our past experiences (Priscu and Goldman 1983a; Priscu and Downes 1984) and the controls included during these experiments (see Materials and Methods section) indicate that physical adsorption of tracer onto cells and filter is negligible. Nevertheless, both adsorption onto cell surfaces and diffusion represent net removal of NH$_4^+$ from the surrounding media, which can be subsequently assimilated, and, from an ecological point of view, be considered uptake (Goldman and Glibert 1982). Finally, isotopic equilibration due to balanced influx–efflux rates seems unlikely considering the substantial increase in ^{15}N atom-% excess observed during the first 5 min of incubation in relation to the small internal NH$_4^+$ pools generally observed in phytoplankton (Goldman and Glibert 1982; Wheeler et al. 1982).

Our measurements of enhanced short-term NO$_3^-$ uptake represent, to our knowledge, the only report of such a phenomenon in natural phytoplankton populations. The very rapid accumulation of ^{15}NO$_3^-$ in particulate material that we observed during the first 5 min implies that, as discussed above for NH$_4^+$, physical processes may be partially responsible. However, unlike NH$_4^+$, large pools of NO$_3^-$ have been shown to accumulate within the cells in both laboratory cultures (e.g. De Manche et al. 1979; Dortch et al. 1979) and natural populations (Collos and Slawyk 1976) of marine phytoplankton. This pool, although variable, can account for up to 55% of the total cell N (De Manche et al. 1979). Therefore, rapid isotopic equilibration due to balanced influx–efflux rates may be partially responsible for the initial rapid accumulation of ^{15}NO$_3^-$ observed in our study. It is important to note that because we added metallic Cu to our Dumas reagents any intracellular NO$_3^-$ should be reduced to N$_2$ gas during combustion and subsequently be measured by emission spectrometry.

The enhanced ability of the Lake Taupo phytoplankton to utilize NH$_4^+$ and NO$_3^-$ during very short exposure times could conceivably allow the phytoplankton to exploit microzones of elevated DIN levels and hence obtain adequate N to maintain growth at high rates despite low ambient DIN and biomass concentrations. This argument is well documented for NH$_4^+$ in marine systems (e.g. Goldman et al. 1979; Horrigan and McCarthy 1982; Goldman and Glibert 1982) and has been invoked for several freshwater systems (Axler et al. 1981; McCarthy et al. 1982; Priscu and Goldman 1983a). Such reasoning appears sound for NH$_4^+$ because it is regenerated rapidly and may appear irregularly as products of animal

TABLE 5. Percent ^{14}C incorporation into the major end products of photosynthesis during 3–5 h incubations at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (L) and in darkness (D). Water for these fractionations was subsampled from the bottles used to determine ρC (see Table 4). LMW = low molecular weight metabolites.

	% ^{14}C incorporation							
	Lipid		Polysaccharide		Protein		LMW	
Date	L	D	L	D	L	D	L	D
7 July 1982	12.0	12.7	40.8	18.3	36.1	63.1	11.1	5.9
3 Aug. 1982	14.5	9.9	53.6	42.7	12.2	31.9	19.7	15.5
26 Aug. 1982	16.8	29.7	37.5	35.9	17.6	18.2	28.1	16.2
Mean ± SD	14.4±2.4	17.5±10.7	44.0±8.5	32.3±12.6	22.0±12.5	37.7±23.0	19.6±8.5	12.5±5.8

TABLE 6. As in Table 5 except that ambient (A) water samples are compared with NH_4^+-enriched (N = +100 μg NH_4^+–N·L^{-1}) samples. The experiment was conducted on 22 July 1982 using near surface water. *Significant from ambient samples at $p < 0.05$ as determined by a t-test.

	% ^{14}C incorporation							
	Lipid		Polysaccharide		Protein		LMW	
	A	N	A	N	A	N	A	N
Light	24.9±2.9	25.1±0.3	41.5±1.3	39.0±1.1	17.4±0.9	23.2±1.5*	16.1±0.8	12.7±0.6*
Dark	12.3±2.3	14.2±0.9	29.2±2.7	35.0±2.6	29.6±8.1	28.9±1.4	29.0±9.9	22.0±3.8

excretion and microbial action (McCarthy and Goldman 1979; Axler et al. 1981). However, NO_3^- is considered a "new" form of DIN (Dugdale and Goering 1967) which is confined primarily below the euphotic zone. It generally enters the euphotic zone periodically with massive disruptions of the thermocline (i.e. upwelling, autumn turnover) and continuously via slow exchange across the thermocline. Dispersion dynamics in pelagic waters are such that pulses of regenerated NH_4^+ and PO_4^{3-} released by zooplankton persist only briefly (Jackson 1980; Lehman and Scavia 1982a, 1982b, 1984) whereas NO_3^- introduced into the euphotic zone can persist for long periods relative to the turnover time of the phytoplankton (Turpin et al. 1981). Thus, whereas the presence of rapid NH_4^+ uptake as a mechanism of maintaining primary productivity under N-deficient conditions is fairly obvious, the ecological significance of rapid NO_3^- uptake is not clear. Vincent and Downes (1981) observed that the highest rate of planktonic nitrification occurred in the epilimnion of Lake Taupo, which led them to suggest that the resultant NO_3^- may be important to the primary productivity in this zone of the lake. Perhaps the enhanced NO_3^- uptake that we observed is a response to microzones of nitrification. Until we overcome our technological limitations in measuring both nutrient micropatches and their rate of generation and utilization, our discussion, as well as those of others, remains speculative.

N Uptake Kinetics

Our NH_4^+ and NO_3^- uptake kinetics experiments revealed relatively low half-saturation constants that are consistent with those calculated in other N-deficient systems (reviewed by Dugdale 1976; see also Axler et al. 1982), implying a high affinity for DIN, particularly NH_4^+. Also in accordance with the information provided in the above references are the higher V_{max} values observed for NH_4^+ uptake relative to NO_3^-. We emphasize that these kinetic constants were obtained from 3–5 h incubations and reflect an integrated physiological response over this period (i.e. they are based on a single time interval and neglect variations in uptake with time). Kinetic parameters obtained from experimental incubations greater than about 1 h probably reflect N assimilation and incorporation rather than simply membrane transport (Wheeler et al. 1982). Consequently, the kinetic parameters reported in our study, as well as the majority of those reported in the literature, tend to overestimate K_s and underestimate V_{max} for the membrane transport system that regulates uptake during periods of enhancement (Wheeler and McCarthy 1982).

Before such differences in the uptake kinetics of phytoplankton can be resolved, one must first be able to measure accurately the nutrient concentration. Errors in the determination of nutrient concentration, such as we report in this study, pose serious problems for calculating the uptake of P in freshwater (Rigler 1966; Lean and White 1983) and of N, both in freshwater (Liao and Lean 1978; Murphy 1980) and the sea (Eppley et al. 1977).

Pathways of C Assimilation

The flow of ^{14}C into the polysaccharide, lipid, protein, and low molecular weight metabolite fractions varied with photon flux density and nutrient level. Attempts by various authors to use the metabolic patterns of C incorporation as an indicator of nutrient status of algae have yielded conflicting results. Collyer and Fogg (1954) and Fogg (1956) observed high rates of lipid synthesis in N-starved freshwater algal cultures whereas Morris et al. (1974) observed an increased proportion of ^{14}C into the protein fraction in N-deficient algae. However, in the latter study a N source was present during part of their incubation; thus, their cultures may have been in a transient state between N limitation and N sufficiency. In one of the few studies

on the pathways of C incorporation in natural populations of freshwater phytoplankton, Priscu and Goldman (1983b) observed no obvious changes in the patterns of ^{14}C incorporation for N-deficient or N-enriched samples in experiments conducted both in vitro and in the framework of a whole-lake NH_4NO_3 enrichment. When Lake Taupo samples were enriched with NH_4^+ and incubated at $100\ \mu E \cdot m^{-2} \cdot s^{-1}$ a significant increase of ^{14}C in the protein fraction relative to unenriched samples was observed. This increase occurred primarily at the expense of ^{14}C incorporation into the polysaccharide and low molecular weight metabolite fractions, indicating that these fractions contain precursors of protein synthesis that require a supply of N to complete the reaction. The decrease of polysaccharide may also partially result from enhanced respiration during N assimilation.

Whenever unenriched samples were incubated in the dark, the relative rate of protein synthesis increased at the expense of ^{14}C flow into the polysaccharide and low molecular weight metabolite fractions. This effect was similar to what we observed in the light-incubated, N-enriched samples. Therefore, as cautioned by Priscu and Goldman (1983b), if the pattern of ^{14}C incorporation into protein is to be used as an index of N limitation, the photon flux density of incubation is critical and must be considered. Furthermore, when a high incorporation of ^{14}C into storage products at the expense of protein occurs, such as that noted in our light experiments, important questions are raised about the growth of such organisms. Our data from Lake Taupo indicate that the extrapolation of midday measurements of ^{14}C uptake to phytoplankton growth would be most accurate if incubation times included a full light–dark cycle, although potential errors with prolonged incubations are well known (Peterson 1980).

While the overall patterns of ^{14}C incorporation can be difficult to relate to the nutritional state of phytoplankton, it appears that the relative synthesis of protein may be a useful index of N uptake. Our estimates of N uptake based on the percent ^{14}C incorporation into protein were remarkably similar to those obtained from ^{15}N-tracer techniques. That the tracer measurements were about 1.4 times greater than the protein-based values may be due to the fact that our ^{15}N uptake rates were not corrected for the effects of isotope dilution (Glibert et al. 1982). Conversely, this discrepancy may also reflect improper assumptions concerning the percentage of phytoplankton N that is protein. We feel that time-course measurements made with this technique, if done in conjunction with time-course N uptake measurements, could add significantly to our knowledge of N transport and assimilation in phytoplankton.

Ecological Considerations

We consider that regenerated NH_4^+ is the primary source of inorganic N for phytoplankton growth in the upper euphotic zone of Lake Taupo. That the NH_4^+ concentration in this zone of the lake was low and relatively constant, and has been shown to be so throughout the year (White et al. 1980), indicates that regeneration rates of NH_4^+ must be similar to uptake rates. The overall maintenance and any seasonal increases in algal biomass presumably result from advected or diffused N as NO_3^-. Thus, the system is in a quasi-steady state in which the pools of NH_4^+ and NO_3^- remain low and constant owing to a balance between N uptake and N supply. It seems, therefore, that the epilimnetic phytoplankton community in Lake Taupo is functionally similar to that of oligotrophic marine systems. In particular, the ability of freshwater phytoplankton to utilize nitrogenous nutrients that may appear in microscale patches has now been shown.

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Genetic Effect on the Dynamics of a Model of Pink (*Oncorhynchus gorbuscha*) and Chum (*O. keta*) Salmon

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Smoker, W. W. 1984. Genetic effect on the dynamics of a model of pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon. Can. J. Fish. Aquat. Sci. 41: 1446–1453.

Different stock dynamics result from genetic and nongenetic mechanisms of determination of maturation age of chum salmon (*Oncorhynchus keta*) in a model of interacting pink (*O. gorbuscha*) and chum salmon stocks. When the model is disturbed from equilibrium by low survival in one pink salmon line, the genetic mechanism (high heritability of maturation age) leads to biennial cycles of numbers of even-aged chums and of numbers of pinks, similar to observed cycles. The nongenetic mechanism (zero heritability of maturation age) results in a new equlibrium at which neither stock cycles. When one pink salmon line is completely removed the genetic mechanism leads to biennial cycles of abundance of even-aged chums; the nongenetic mechanism does not lead to such cycles. These effects persist at intermediate values of heritability of maturation age and in spite of stochastic variability. The model is an adaptation of the Ricker curve to two interacting stocks, the recruitment for each depending on the density of both.

Dans un modèle d'interaction entre le saumon kéta (*Oncorhynchus keta*) et le saumon rose (*O. gorbuscha*), des mécanismes génétiques et non génétiques déterminant l'âge de maturation du saumon kéta ont des effets sur la dynamique des stocks de ces deux espèces. Quand l'équilibre du modèle est modifié par une faible survie chez l'une des lignées de saumon rose, le mécanisme génétique (héritabilité élevée de l'âge de maturation) est la cause de cycles bisannuels du nombre de kétas de même âge et du nombre de roses, de façon semblable aux cycles observés. Le mécanisme non génétique (absence d'héritabilité de l'âge de maturation) provoque l'apparition d'un nouvel équilibre où les cycles des stocks sont absents. Quand une lignée du saumon rose est complètement éliminée, le mécanisme génétique provoque l'apparition de cycles bisannuels de l'abondance de kétas de même âge ; le mécanisme non génétique ne donne pas naissance à de tels cycles. Ces effets persistent aux valeurs d'héritabilité intermédiaires, même en dépit de la variabilité stochastique. Ce modèle est une adaption de la courbe de Ricker pour deux stocks en interaction, le recrutment de chacun des stocks étant fonction de la densité des deux.

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Chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon share similar life cycles and ecologies. They spawn in late summer and fall and migrate to sea immediately on emergence from spawning gravels in the spring. Pink salmon spawn virtually exclusively at 2 yr of age in their natural range; chum salmon generations overlap because they spawn at ages from 2 to 6, mostly from 3 to 5. The species commonly cooccur during spawning and during early sea life.

Maximum survival from generation to generation at intermediate stock sizes has been observed in chums (Ricker 1975; Helle 1979) and pinks (e.g. Merrell 1962). Maximum production of fry at intermediate combined stock sizes of cooccurring pink and chum salmon has also been observed (Wickett 1958; Hunter 1959; McNeil 1968). Such density-related production has been attributed to compensatory mortality of embryos (Neave 1953). Such mortality has been attributed to the superposition of redds by late-spawners on those of early-spawners coupled with poorer survival of late-spawned embryos (McNeil 1968). It has also been attributed to accelerated oxygen demand by alevins and decomposers and the resulting suffocation after hatching in overseeded spawning beds (Heard 1978). The relationship of survival to combined numbers in cooccurring stocks of pinks and chums has been attributed to common sources of freshwater mortality (flood scouring, predation), and overlapping spawning beds (Hunter 1959), and may also be due to overlapping demands for limited prey, especially during the onshore stage of early life (Kaczynski et al. 1973; Feller and Kaczynski 1975; Bailey et al. 1975; Gallagher 1979; Landingham 1982).

Pink salmon lines that spawn in alternate years in a stream or area often exhibit a biennial cycle of abundance (Ricker 1962). Ricker concluded that this important feature of pink salmon population dynamics probably results from a set of effects possibly including those of predation, fishing, intraspecific competition, and cannibalism by dominant line adults on fry of the other line. Withler and Morley (1982) suggested that dominant lines may have inherently greater productivity because of a founder effect.

Chum salmon also sometimes exhibit biennial cycles in spite of their overlapping generations. The catch data of Bilton (1973) for British Columbia during 1957–71 reflect such cycles in some production areas. During 1960–69 the ratio of the number of observed chums to the forecast number of chums in

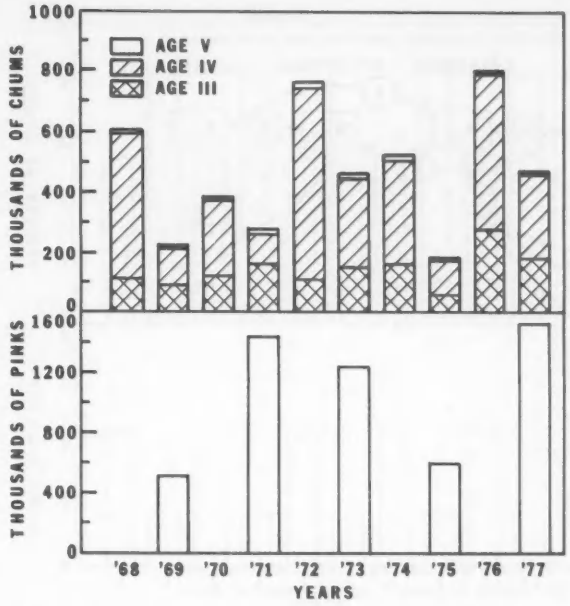

FIG. 1. Numbers of chum and pink salmon returning to Puget Sound, 1968–77. Washington Department of Fisheries data reconstructed from Gallagher (1979).

Johnstone Strait and the Fraser River displayed a biennial cycle (D. Anderson, Department of Fisheries and Oceans, Vancouver, B.C., 1976 Pink and Chum Salmon Workshop, pers. comm.). The average age and abundance of the catch of chums in Puget Sound have had a biennial cycle; the proportion of age IV chums and total number are high during years when pink salmon are not abundant (Washington Department of Fisheries data in Gallagher 1979) (Fig. 1).

Gallagher (1979) suggested that a genetic model might explain these cycles of chum salmon. If in years of pink rarity, chums produce 65% age IV offspring and 35% age III, and 50% of both ages in the alternate years of pink abundance, the observed cycles will result. It is not clear how such a genetic maintenance of separate age distributions by year could be sustained in the face of crossbreeding between cohorts of different ages. That model does not reveal how such a mechanism could evolve, and does not conceptualize interactions between the species, although a causative interaction between pinks and chums is implicit. It is ambiguous concerning environmental or genetic processes as causes for the observed effect (Gallagher 1979, p. 90).

The model proposed here depicts two interacting species, pinks and chums, the production of each of which varies over time. It permits explicit explorations of genetic and nongenetic (environmental) mechanisms that might underlie variation of chum salmon age.

Method

Two-Species Model

Structure

The model considers the production of a stock of pink salmon and a stock of chum salmon in a common spawning and early sea life environment (Fig. 2). A Ricker curve (Ricker 1975, Chap. 11) models the density-dependent production of returning offspring for each stock in a given year:

$$R_c = a_c P_c \exp[-B_c(P_c + K_p P_p) + V_c]$$

and

$$R_p = a_p P_p \exp[-B_p(P_p + K_c P_c) + V_p]$$

where the subscript p refers to the stock of pinks and the subscript c to that of chums. The parameters a, B, R, and P are as in Ricker (1975) except that a and B are used instead of alpha and beta. They are, respectively, the returns per spawner at small stock sizes, the inverse of the stock size that produces maximum returns, the number of returns, and the number of parents (the stock size). The inclusion of the number of parents (P) of both stocks in the density-dependent part of the prediction of the number of returning offspring (R) models the interaction between species. Murphy (1968) first proposed a similar model. The number of each species is the equivalent of a number of the other species as in the classical competition model of Volterra (1926) and Lotka (1932) (both cited in Hutchinson 1978), which is based on the logistic model of population growth. The coefficients (K) are analogous to the competition coefficients in the logistic model. Equilibrium solutions of these equations result in similar competition isoclines to those of the logistic model (Murphy 1968) (Appendix A).

Parameter V is normally distributed with a mean of zero and a specified variance. The model uses a multiplicative, log-normal distribution of random variation (e.g. Peterman 1981 found it to be an appropriate description of variability of return in salmon populations).

Age determination

When the numbers of offspring have been computed for the numbers of parents in a given year, the offspring are apportioned as parents into future years. For the pink salmon, maturation age (II) does not vary, the chum salmon age varies from III to V, and the offspring of a given brood year must be assigned to one of three future years as parents. The chum parents in a given year include members of three brood-year cohorts. This apportionment of chums is by a genetic or a nongenetic (i.e. environmental) mechanism, or by a simple mixture of the two mechanisms, a simplification of what is probably a complex of interactions in nature.

In the simplified genetic mechanism the proportions of offspring of each age at maturation are the same as among the parents (hypothesis 1 of Godfrey 1958; Larkin and Hourston 1964). This is equivalent to the heritability of maturation age being unity. When this is true, offspring have the same mean age as the midvalue of their parents because all variation of maturation age in the stock is due to genetic variation (e.g. Falconer 1981, Chap. 10).

In the nongenetic mechanism the proportions of the offspring having each age at maturation are not determined by their parents, but follow some arbitrary exogenous distribution. None of the variation of maturation age in the stock is due to genetic variation; all variability of maturation age derives from nongenetic (environmental) variability (hypothesis 2 of Godfrey 1958). In this case, heritability of maturation age is nil. The model assigns the same mean distribution of ages to the offspring of every generation; thus, the model does not imitate any systematic change of environmental effects. Random environmental variability is, however, imitated by the model's

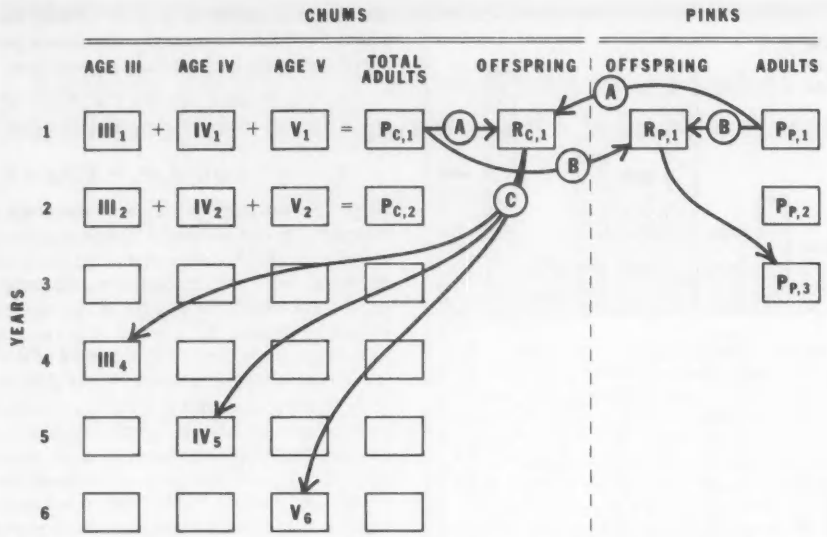

FIG. 2. Two-species density-dependent model of the dynamics of interacting pink and chum salmon. A, recruitment relationship for chums; B, recruitment relationship for pinks; C, apportionment of chums among maturation ages.

log-normal variation of survival; it is mimicked in simulations of either mechanism. Intermediate heritabilities of maturation age model situations in which both environmental and genetic variation contribute to the variability of maturation age. In these cases the model assigns distributions of ages to the offspring that (distributions) are intermediate between those of the parents and the exogenous distribution of ages.

Initial conditions

Simultations generally began with each stock at 5 units, an equilibrium, or at some disruption of that equilibrium. Initial numbers, which varied between simulations, were maintained for the first 2 yr for pinks, the first 3 yr for age III chums, 4 for age IV, and 5 for age V. The model generated numbers in each category in subsequent years.

Simulations

I examined the behavior of this model by varying (1) the mechanism of chum age determination, genetic or not, (2) the initial numbers of fish in each age group and in each stock, and (3) the values of parameters of the Ricker curves. Analysis of the deterministic case (Appendix B) revealed no equilibrium conditions of cycling abundance or age, but in simulations abundance and age patterns changed very slowly (the rate of returns per spawner remained within 0.02 of 1.00) after about 20–30 simulated years. Therefore I examined the behavior of the model at an apparent equilibrium: after 40 simulated years.

First I examined a case in which the only difference between the species was that of their age structures. In both equations the productivity parameters a were set at 2 and the capacity parameters B at 0.069 (i.e. both stocks had an equilibrium size of 10 stock units). Both (K) coefficients were unity, i.e. one chum parent had the same effect on pink returns as one pink parent and vice versa. The model was deterministic, Var (V) = 0. The initial numbers were 5 units for each stock. The simulations began either at equality in both the first and second years or with a pink salmon cycle, having 5 units the first year and 0.5 the second. The age distribution of chums was 1 unit age III, 3 units age IV, and 1 unit age V. I examined the effects of the genetic and nongenetic mechanisms of age determination (heritability one, heritability zero). This required four simulations of combinations of age determination mechanisms with cycling and noncycling pink salmon lines: genetic mechanism with either cycling or noncycling lines and nongenetic with either cycling or noncycling lines.

I then examined the sensitivity of the results of these simulations to changes in the values of the parameters and initial numbers. Still other simulations had different values of the (1) interaction coefficients, K = (0.5, 1), (2) productivity parameters ($a_p = 3$ and $a_c = 2$, $a_p = 2$ and $a_c = 3$), and (3) variance of the random variation term Var (V) = (0, 2). In this last instance the stocks either varied independently or covaried, i.e. they have the same randomly chosen variance term in a brood year.

I examined a second case characterized by an asymmetric distribution of initial chum salmon ages, 2.3 units age III, 2.3 units age IV, and 0.4 unit age V along with the complete absence of one of the pink salmon lines, mimicking the situation in Puget Sound where pinks spawn only in one line (Fig. 1).

To simplify the analysis of the model's behavior I did not examine the full set of combinations of the different values, but only looked for changes of results of the first four simulations brought about by varying each parameter or initial value, holding the others as they were in the first case.

Results

Simulations

In the first simulations the pink salmon did not cycle initially; the only difference between the species was that of their age structure. No change occurred when either the genetic or the environmental mechanism was used to determine the ages of chum salmon spawners. In both instances both stocks remained at the initial numbers. In the case in which the pink salmon did

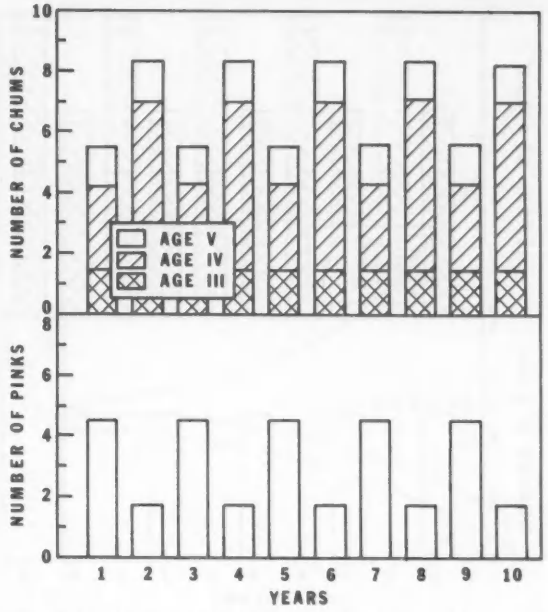

FIG. 3. Simulation of abundances during 41st to 50th year of interacting pink and chum salmon after a 90% reduction in one pink salmon line (5 units in year 1 of simulation and 0.5 in year 2; 1 unit age III chums, 3 units age IV, and 1 unit age V in initial years). Genetic mechanism of age determination (heritability of maturation age is unity).

cycle (odd years began with 5, even years with 0.5) the two mechanisms brought about different changes. The environmental mechanism caused the two stocks to come to a new equilibrium at which the chum salmon stock had 7.1 units and the pink stock 2.9 units every year. The genetic mechanism did not lead to a new equilibrium; the cycle of numbers of pinks was maintained, 4.5 units in the odd years, 1.7 in the even. A cycling of chum abundances also occurred, 5.6 units in the odd years and 8.2 in the even, mostly due to cycling of the numbers of age IV chums (Fig. 3).

Sensitivity to parameter values

The same pattern, the genetic mechanism maintaining biennial cycles of the numbers of age IV chums and of pinks, resulted (1) with an asymmetric initial age distribution of chums, (2) with competition coefficients greater than 0.5, and (3) when the productivity parameter a was 3 in one species and 2 in the other. (The species with the greater value of a required a slightly longer time to achieve apparent equilibrium, 31 versus 18 yr in one extreme instance.) In no modification of the parameter values and initial conditions did the environmental mechanism lead to any cycling of numbers. With equal initial numbers of pink salmon in odd and even years there was no departure from the initial conditions when either the environmental or genetic mechanism was used to determine chum salmon age.

If one pink salmon line was entirely absent (second case) both the genetic and the environmental mechanism caused biennial cycling in the numbers of chum salmon. The environmental mechanism resulted in small cycles in numbers of age III and age IV chums which were equal and opposite in odd and even years but no cycle in total chum abundance (Fig. 4a). The genetic mechanism produced a biennial cycle in numbers of age IV chums (and in the total number of chums) that was equal and opposite to the biennial cycle of pink salmon numbers in the one present line (Fig. 4b).

Sensitivity to value of heritability

Intermediate values of heritability of maturation age also resulted in biennial cycles of numbers of even-age chums and of pinks, but the cycles were reduced in amplitude; the relationship between the cycle amplitudes and heritability is not linear, the amplitudes being more reduced at low values of heritability. (Fig. 5).

Sensitivity to random variation

For stochastic simulations $\mathrm{Var}(V) > 0$, I computed for each age-class and stock the average difference between the numbers in the odd and even years during the 41st through the 60th years, then the mean and variance of 30 such averages taken from independent simulations in which $\mathrm{Var}(V)$ was drawn from the same distribution (mean of zero and variance which differed between simulations, $0.1 < \mathrm{Var}(V) < 1.3$), and then tested the significance of the difference of this mean from zero with a t-test. This gave an approximate description of the amount of random variation that would obscure the effect of the genetic mechanism.

Under the genetic mechanism, when the two species varied independently, variances of 0.4 or smaller resulted in significantly different numbers of age IV chums in the odd and even years (these differences were greater than 1 stock unit) (Table 1). There was similar evidence of cycling (odd–even differences) for the pinks. These differences were opposite in sense for the age IV chums and for the pinks. When the difference for chums was positive, the difference for pinks was negative. When the two species covaried, variances of about 1.0 or smaller resulted in significant cycling of age IV chums and of pinks (Table 2). Under the environmental mechanism and only one pink line, variances less than 0.4 produced significant cycles of numbers of chums in odd- and even-age groups but these were roughly equal and opposite to one another and were less than 1 stock unit (Table 3).

For intermediate heritabilities the critical levels of variability (above which cycles were not significant) were reduced, moreso when the stocks varied independently (Table 4).

Discussion

In this model system in the presence of biennially cycling pink salmon a high heritability of maturation age can result in a biennial cycle of the age structure of the chums (numbers of even-aged spawners), a cycle of the total abundance of chum spawners, and maintenance of the biennial cycle of pinks. Low heritability of maturation age results in biennial cycles of numbers of chums only when one pink salmon line is entirely missing. The latter small cycle (less than one unit) is due to the recurring reduced survival of chums in biennial competition with pinks; both the odd- and even-aged groups cycle. Total chum abundance does not cycle. The former cycle is an adaptation of the chum stock to interaction with pinks. Part of the stock's production is removed from competition in that even-aged chums spawn predominantly in the pinks' off-cycle years.

The heritability of age of maturity is not known in any chum

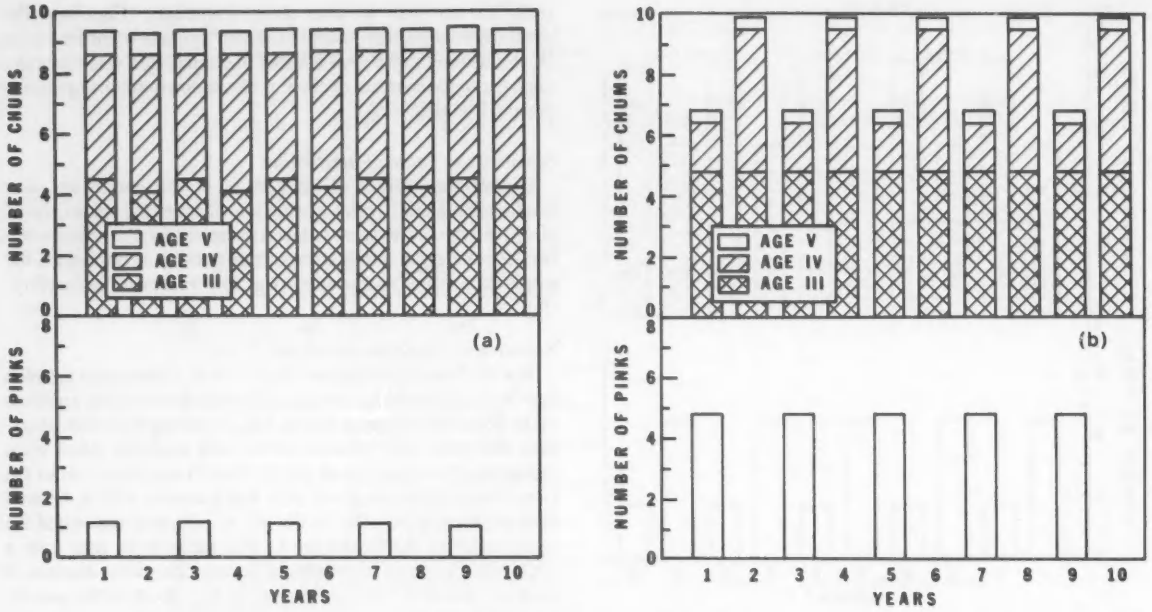

FIG. 4. (a) Simulated abundances during 41st to 50th year of interacting pink and chum salmon when only one pink salmon line is present (5 units in year 1 of simulation, 0 in year 2; 2.3 units age III chums, 2.3 units age IV, and 0.4 unit age V). Environmental mechanism of age determination (heritability of maturation age is zero). (b) Simulated abundances. Environmental mechanism of age determination (heritability of maturation age is unity).

stock. There is some evidence that the heritability of age of maturity is significant in some populations of salmonids. Piggins (1974) found a correlation of sea ages between Atlantic salmon (*Salmo salar*) parents and their offspring. Gjerde and Gjedrem (1984) estimated the heritability of maturation age in a stock of Atlantic salmon to be 0.39 by sire component and 0.49 by dam component. Gjerde and Gjedrem (1984) estimated the heritability of maturation age in a stock of rainbow trout (*S. gairdneri*) to be 0.21 by sire component and 0.26 by dam component. Milne (1955) and Godfrey (1958) demonstrated a correlation between ages of sockeye salmon (*O. nerka*) parents and their offspring in two British Columbia stocks, which is evidence that heritability of maturation age may be significant in those stocks.

Ellis and Noble (1961) reported the age of maturation of the offspring of crosses between age II male chinook salmon (*O. tshawytscha*), age II males, and age III females in the Deschutes River stock. The average age of the offspring of the age II males was 2.3 y for males and 3.7 yr for females; the average age of offspring of the age III males was 3.15 yr for males and 3.9 yr for females. Taking the midpoints between the average ages of the sexes in the offspring and regressing them on the midpoints of the parent ages gives a slope (heritability) of 0.24. Ricker (1981) made a similar estimate for the chinook stock at the University of Washington, getting a value of 0.25–0.3. These estimates are evidence that heritability of maturation age can be significant in some stocks of salmonids, but the estimates are no indication of typical values in chum salmon stocks.

Alternate-year interaction with pinks and reduced productivity in those alternate years might also serve to enhance the genetic variability of maturation age (and thus increase its heritability) in a stock of chums because spawners of different ages would tend to be segregated temporally and would tend to mate assortatively with respect to age. If the breeding values of spawners (the difference between the mean value, age, of the spawner's offspring and the mean value in the population; Falconer 1981) are significantly correlated, the additive genetic variance of maturation age would be increased in the stock by this assortive mating. Heritability of maturation age might thus be higher in a chum stock that is affected by a cycle of pink numbers and lower in a stock that is not so affected.

Intermediate heritabilities in this model are unrealistic in that the age distribution in the entire population (spawners and nonspawners) does not change. In each year the age distribution

TABLE 1. Mean of average difference between numbers of fish in odd and even years, 41st through 60th years of 30 independent simulations. Case in which pinks cycle in initial years. Genetic mechanism of chum age determination. Different levels of log-normal variation, species vary independently. **Significantly different from 0, $P < 0.01$; *$P < 0.05$.

	Chums			
Variance	Age III	Age IV	Age V	Pinks
0.1	−0.004	−2.700**	−0.016	2.752**
0.2	0.002	−2.361	0.023	1.976**
0.3	0.024	−2.172**	−0.023	2.670**
0.4	0.009	−2.149**	−0.009	2.466**
0.5	0.048	−1.017	0.038	1.024
0.6	0.082	−1.333*	0.179	0.957
0.7	−0.091	−0.620	−0.103	0.719
0.8	0.093	0.292	−0.162	0.406

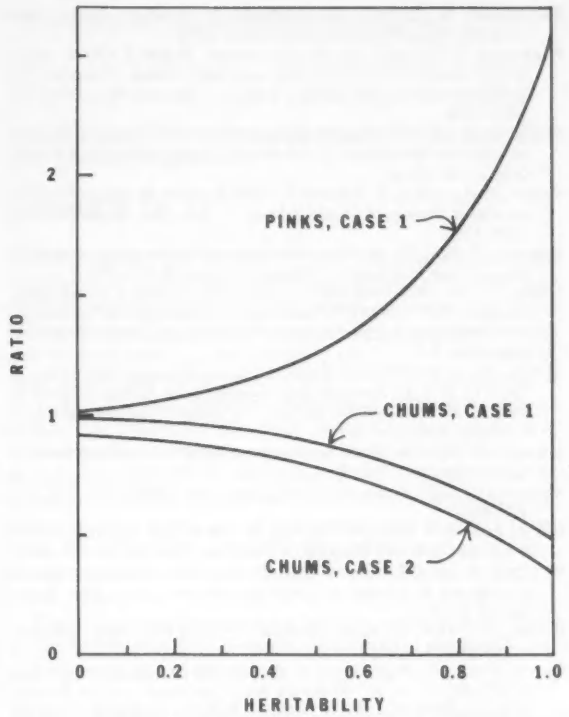

FIG. 5. Ratio of numbers (stock units) of pink salmon and of age IV chum salmon in odd years to numbers in even years as a function of the heritability of maturation age of chums (41st through 60th years of simulations). Case 1, simulation began with a pink salmon cycle, 5 units in first year, 0.5 in second, and with 1 unit age III chums, 3 units age IV, and 1 unit age V. Case 2, simulation began with 5 units of pink salmon in first year, none in second, 2.3 units age III chums, 2.3 units age IV, and 0.4 unit age V.

TABLE 2. Mean of average differences when species covary. **Significantly different from 0, $P < 0.01$; *$P < 0.05$.

	Chums			
Variance	Age III	Age IV	Age V	Pinks
0.4	−0.013	−2.583**	−0.038	2.266**
0.6	−0.265	−1.500**	−0.101	2.032**
0.7	−0.222	−1.303**	−0.403	1.840**
0.9	−0.358	−1.632**	−0.978	1.678**
1.0	−0.517	−0.948	0.157	1.889**
1.1	2.387	−2.113**	1.542	0.451
1.2	0.781	−2.698**	0.458	−0.266
1.3	0.270	−0.906	0.644	0.464

of spawners is compared with the same exogenous distribution. It is not clear which collection of age groups and cohorts should realistically be compared with the spawners in a given year; unreported simulations in which I compared the age distribution, for instance, of all fish born in the same years as spawners in a given year lead to results similar to those reported above.

There is no evidence on the magnitude of interaction coefficients that apply to cooccurring pink and chum stocks in nature. If they are smaller than about 0.5 the model predicts that

TABLE 3. Mean of average differences in the case of only one pink line and in which chum ages are environmentally determined. Different levels of log-normal variation, species vary independently. **Significantly different from 0, $P < 0.01$; *$P < 0.05$.

	Chums			
Variance	Age III	Age IV	Age V	Pinks
0.1	0.423**	−0.418**	0.069**	1.244**
0.2	0.355**	−0.368**	0.072**	0.302**
0.3	0.429**	−0.415**	0.074**	1.023**
0.4	0.512**	−0.508**	0.092**	0.998**
0.5	0.258	−0.222	0.040	1.422**
0.6	0.299	−0.437**	0.052	0.657**
0.7	−0.209	−0.268	0.049	0.815**

TABLE 4. Critical values of log-normal variance, above which significant cycles of numbers of even-age chums and of numbers of pinks did not occur, for intermediate values of heritability of maturation age of chums. Case in which pink salmon cycled initially (5 units in the odd year and 0.5 in the even year).[a]

Heritability of maturation age	Independent stocks	Covarying stocks
0.1	<0.1	<0.1
0.2	0.1	0.2
0.3	0.1	0.2
0.4	0.1	0.4
0.5	0.1	0.5
0.6	0.2	0.6
0.7	0.3	0.7
0.8	0.4	0.9
0.9	0.6	0.9
1.0	0.8	1.0

[a]Discrepancies with Tables 1 and 2 arise because Table 4 was generated by an independent set of simulations; by chance, higher critical values emerged for the independent stocks.

the genetic mechanism would produce no remarkable effect. Their relative sizes and fecundities suggest that the effect of chums on pinks would be greater than the effect of pinks on chums, but both effects would have to be significant if the predicted effects of the model were to come about. Gallagher (1979) did present evidence that the production of chums or of pinks can be significantly affected by the presence of the other species during early life stages.

High variability of recruitment can erase the effect of the genetic mechanism. It is not clear which form of variability, independent or covarying, is more nearly realistic. The critical level of variability, above which the effect of the genetic mechanism is lost, is higher if the stocks covary. Most mortality for both species occurs during the early stages when they cooccur and would tend to cause the stocks to covary. Unusual catastrophic flood scouring, for example, would kill the embryos of both stocks. Observed variances range between 0.25 and 0.49 for chums and between 0.23 and 0.96 for pinks in British Columbia production areas (F. Wong, Pacific Biological

Station, Nanaimo, B.C., pers. comm.). If variances were known for discrete stocks, they might be smaller. The critical values in the model are about 0.4 and 0.9 for the independent and covarying cases (Tables 1 and 2) if heritability is unity and are reduced for intermediate values of heritability (Table 4). Observed variances, however, are below some of the critical values; natural variability of recruitment would not necessarily erase the effect.

The strongest suggestion that a genetic mechanism, like the one modelled here, operates to adapt chums to pink cycles is in the Washington Fisheries Department data in Gallagher (1979) (Fig. 1). If an environmental mechanism were controlling the distribution of chum ages, both the number of age III and the number of age IV chums would cycle biennially and there would be no cycle of the total number of chums (Fig. 4a). The observed data (Fig. 1) are most similar in pattern to that predicted by the genetic mechanism (Fig. 4b).

Genetic adaptation of chums to biennial cycling of pinks is plausible as a mechanism underlying biennial cycles in age structure and numbers of chums and for subsequent maintenance of those cycles in pink salmon abundance caused, for instance, by a single extraordinary failure of one line.

Acknowledgments

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Appendix A

In these equations the parameters are defined as they are in the text (above). Equations (1) and (2) describe the productivity of interacting chum and pink stocks; they take no account of the different age structures of the two stocks (see Appendix B). These equations result in competition isoclines similar to those of the classical competition model based on a logistic model of population growth (Murphy 1968):

$$\text{(A.1)} \quad R_c = a_c P_c \exp\left[-B_c(P_c + K_c P_c)\right]$$

$$\text{(A.2)} \quad R_p = a_p P_p \exp\left[-B_p(P_p + K_c P_c)\right]$$

$$\text{(A.1a)} \quad \ln(a_c) = B_c(P_c + K_p P_p) \quad \text{when } R_c = P_c$$

$$\text{(A.2a)} \quad \ln(a_p) = B_p(P_p + K_c P_c) \quad \text{when } R_p = P_p$$

$$\text{(A.1b)} \quad P_c = [\ln(a_c)]/B_c - K_p P_p$$

competition isocline, slope $= -K_p$

$$\text{(A.2b)} \quad P_p = [\ln(a_p)]/B_p - K_c P_c$$

competition isocline, slope $= -K_c$.

The equilibrium population sizes are given by

$$(A.3)\quad P_c^* = [\ln(a_c)/B_c] - [K_p \ln(a_p)/B_p]/(1 - K_pK_c)$$

$$(A.4)\quad P_p^* = [\ln(a_p)/B_p] - [K_c \ln(a_c)/B_c]/(1 - K_pK_c)$$

where P_c^* and P_p^* are the equilibrium population sizes of the chum and pink populations.

Appendix B

Here I address the question of whether, at equilibrium, the numbers of modelled chum salmon depend in a predictable way on the numbers of modelled pink salmon, and vice versa. In the following equations the parameters are as defined above except that the subscripts i, j, and k refer to species ($i = c$ for chums, p for pinks), age (of chums, j = III, IV, V), and year (k = 0, 1, 2, ...). A dot subscript indicates a summation over all values. The parameter A_{cj} is the product of the parameter a_c defined earlier and the proportion of chum salmon that spawn at age j (q_j) ($A_{cj} = A_c \times q_j$). First I solve by iteration the equation that relates the ratio between numbers of chums in odd and even years to the ratio of numbers of pinks in odd and even years. Then I take the partial derivative of the numbers of chums in odd or even years with respect to the numbers of pinks in those years in order to find extrema in the numbers of chums.

The total return of chums in a year is the sum of age III salmon produced 3 yr previously, age IV produced 4 yr previously, and age V produced 5 yr previously:

$$(B.1)\quad R_{cjk} = \Sigma_{jk} A_{cj} P_{c\cdot k-j} \exp -B_c(P_{c\cdot k-j} + K_pP_{pk-j}).$$

The total return of pinks in a year is given by

$$(B.2)\quad R_{p\cdot k} = a_p.P_{p\cdot k-2} \exp -B_p(P_{p\cdot k-2} + K_cP_{ck-2}).$$

Assuming that the two pink salmon lines are each at constant population size, not necessarily the same size, it is true that the number of pinks returning in a year is the same as the number returning 2 yr previously or later (note that $P_{i\cdot k} = R_{i\cdot k}$):

$$P_{p\cdot 0} = P_{p\cdot 2} = P_{p\cdot 4} \cdots$$

and

$$P_{p\cdot 1} = P_{p\cdot 3} = P_{p\cdot 5} \cdots$$

and $P_{p1} = MP_{p0}$ where M is the ratio of the number of odd-year pinks and the number of even-year pinks.

It is also therefore true that the number of chums spawning in a year is the same as the number spawning 2 yr previously or later:

$$P_{c\cdot 0} = P_{c\cdot 2} = P_{c\cdot 4} \cdots$$

and

$$P_{c\cdot 1} = P_{c\cdot 3} = P_{c\cdot 5} \cdots$$

and $P_{c1} = NP_{c0}$ where N is the ratio of the numbers of chums in odd and even years.

Under these conditions the number of chums spawning in an even or odd year, k, is (from eq. B.1)

$$(B.3)\quad P_{c\cdot k} = (A_{c5} + A_{c3})P_{c\cdot k-1} \exp -B_c(P_{c\cdot k-1} + K_pP_{p\cdot k-1}) + A_{c4}P_{c\cdot k} \exp -B_c(P_{c\cdot k} + K_pP_{p\cdot k})$$

and (from eq. B.2)

$$(B.4)\quad P_{c\cdot k} = [\ln(a_p) - B_pP_{p\cdot k}]/B_pK_c.$$

Thus (from eq. B.3 and B.4)

$$(B.5)\quad P_{c\cdot k} = A_{c\cdot}[\ln(a_p)]/B_pK_c + (A_{c4}/K_c - A_{c\cdot}/K_c)P_{p\cdot k-1} \times \exp[P_{p\cdot k-1}B_c(1 - K_cK_p)/K_c] \times \exp[-B_c \ln(a_p)/B_pK_c] - (A_{c4}/K_c)P_{p\cdot k} \times \exp[P_{p\cdot k}B_c(1 - K_cK_p)/K_c] \exp[-B_c \ln(a_p)/B_pK_c].$$

Substituting $P_{c1} = NP_{c0}$:

$$(B.6)\quad N = 1 + [\ln(1 - q_4N - q_4) - \ln(N^2 - q_4N - q_4N^2)]/B_cK_p[K_cP_{c0} - (P_c)/K_p)]$$

which has no explicit solution. (The proportion of chums spawning at age IV is q_4 where $q_4 = 1 - q_3 - q_5$.)

I solved this expression for N, the ratio of chums in odd years to chums in even years, by estimating N with values chosen from the intervals [0, 1) and (1, 10] and iteratively evaluating the right-hand side of the equation, substituting the value for each iteration for N in the next iteration until the values of succeeding iterations did not differ by more than 0.001. I obtained solutions for values of P_{c0} chosen from the interval $(1/4B_c, 1/B_c)$, for values of $P_{p0} = (0, 4/3B_p)$, for values of K_c and K_p from the interval [0.8, 1), and for values of q_4 from [0, 1). These values represent realistic and interesting values for the model. B_c and B_p were 0.07, i.e. the stock size that would produce the maximum number of returns by either species in the absence of the other was about 14 stock units.

The only cases in which there were convergent positive values for N and positive values for M were cases in which q_4, N, and M were all unity, i.e. in which all chums were evenaged and there were no differences in numbers (cycles) from year to year. It appears that there are not interesting solutions (different, realistic numbers of pinks and chums in odd and even years) under the assumption of equilibrium: constant numbers of even-year pinks and of odd-year pinks.

The instantaneous rate of change of the number of chums of even or odd age, k, with respect to the number of pinks of the same age, k, is

$$\partial P_{ck}/\partial P_{pk} = -(a_{c4}/K_c) \exp[-B_c \ln(a_p)/B_pK_c] \times [1 + P_{pk}B_c(1 - K_cK_p)/K_c] \times \exp[P_{pk}(B_c(1 - K_cK_p)/K_c]$$

and the instantaneous rate of change with respect to the number of pinks of the alternate age, $k - 1$, is

$$\partial P_{ck}/\partial P_{pk-1} = (1/K_c)(a_{c4} - a_{c\cdot}) \times \exp[-B_c \ln(a_p)/B_pK_c] \times [1 + P_{pk-1}B_c(1 - K_cK_p)/K_c] \times \exp[P_{pk-1}(B_c(1 - K_cK_p)/K_c].$$

The former instantaneous rate is zero when $a_{c\cdot} = a_{c4}$, i.e. when none of the chums mature at odd-numbered ages, or when the number of alternate-age pinks is $P_{p\cdot k-1} = -K_c/B_c(1 - K_cK_p)$, which is negative if K_c and K_p are in (0, 1), the most interesting values. The latter instantaneous rate is zero when $a_{c4} = 0$, i.e. all of the chums mature at odd-numbered ages, or when the number of like-age pinks is $P_{p\cdot k} = -K_c/B_c(1 - K_cK_p)$, which is again a negative value for interesting values of K. Thus, the productivity of chums is extreme, with respect to number of pinks, only in the unrealistic cases in which the ages of chums are either all odd or all even under the assumption of equilibrium numbers of pinks in odd and even years.

Spawning Characteristics of Sympatric Populations of Steelhead Trout (*Salmo gairdneri*): Evidence for Partial Reproductive Isolation

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We differentially tagged upstream migrant adult summer and winter steelhead trout (*Salmo gairdneri*) of hatchery and wild origin in the Kalama River, Washington, and subsequently observed them spawning under natural conditions in a tributary. We found that summer steelhead spawned within the study stream on a spatially random basis. The steelhead spawning interval was 6 mo long, during which hatchery summer steelhead spawned first, followed by wild summer, hatchery winter, and wild winter steelhead. Significant temporal spawning differences were found between hatchery summer and wild winter fish, and between wild summer and wild winter steelhead. We developed estimates of potential reproductive overlap between spawner groups that indicated that most reproductive activity involved members of the same spawner group. Estimated genetic exchange among groups was primarily between hatchery and wild fish within races. Of all possible spawner group comparisons, the smallest potential reproductive overlap estimated was between wild summer and wild winter steelhead. We concluded that, although wild summer and wild winter steelhead were not completely reproductively isolated, a substantial degree of successful gene flow restriction occurred, thereby providing a mechanism for the maintenance of racial identity. Additionally, spatial and temporal spawner segregation was of insufficient magnitude to prevent the potential infusion of hatchery steelhead genes into the wild summer steelhead population.

Nous avons marqué des truites arc-en-ciel (*Salmo gairdneri*) adultes de deux groupes, truites d'hiver et truites d'été, nées en pisciculture ou à l'état sauvage, au cours de leur migration d'amont dans la rivière Kalama (Washington). Nous avons ensuite observé leur frai en conditions naturelles dans un tributaire. Les frayères des truites d'été variaient de façon aléatoire dans le cours d'eau étudié. La période de frai a duré 6 mo ; les truites de pisciculture d'été frayaient les premières et étaient suivies par les truites sauvages d'été, les truites de pisciculture d'hiver et les truites sauvages d'hiver. Des écarts significatifs entre les périodes de frai ont été notés entre les truites de pisciculture d'été et les truites sauvages d'hiver et entre les truites sauvages d'été et les truites sauvages d'hiver. Nous avons estimé les recouvrements reproductifs possibles des groupes de frai ; la plus importante activité reproductrice était notée entre les membres d'un même groupe. Les échanges génétiques entre les groupes s'effectuaient surtout entre les truites de pisciculture et sauvages de même races. Pour tous les groupes de frai, le recouvrement reproductif possible le moins important était celui des truites sauvages d'été et des truites sauvages d'hiver. Nous concluons que même si les truites arc-en-ciel sauvages d'été et d'hiver ne sont pas totalement isolées du point de vue reproductif, leurs échanges génétiques n'en sont pas moins limités de façon appréciable, ce qui représente un mécanisme pour le maintien de l'identité raciale. De plus, la ségrégation spatiale et temporelle des reproducteurs n'était pas suffisamment importante pour prévenir un transfert des gènes des truites de pisciculture vers les truites sauvages d'été.

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The existence of discrete sympatric populations presents a significant challenge to those involved in management of fish populations. When species are segregated into distinctly different reproductively isolated or ecologically unique units, then proper and effective management requires an understanding of the extent and qualities of those differences.

For salmonids, reproductive isolation in sympatry has been examined for a number of species (Svärdson 1970; Behnke 1972; Allendorf et al. 1976; Goodier 1981). The maintenance of stock discreteness in salmonids occurs largely through spatial isolation mechanisms (natal homing and distance) and/or temporal isolation mechanisms (spawning time) (Biette et al. 1981; Horrall 1981; MacLean and Evans 1981). Disruption of preexisting isolating mechanisms (i.e. through selective harvest, or successful intraspecific hybridization with geneti-

cally different stocks) may eventually lead to a loss of stock genetic identity (Helle 1981), reduced long-term production, and overall fitness (MacLean and Evans 1981).

In the Pacific Northwest, steelhead trout (*Salmo gairdneri*) are commonly managed as two separate stocks or "races" (Withler 1966; Everest 1973). Though some degree of overlap often occurs, summer and winter steelhead races are distinguished primarily by their relative tendencies to return to freshwater at different times of the year on their spawning migration. Summer steelhead typically return as relatively sexually immature adults between the months of May and September, whereas the more sexually mature winter steelhead typically return between November and April (Smith 1960; Withler 1966). Both groups spawn in the late winter and spring. Where they occur sympatrically, steelhead may often be found entering freshwater in all months of the year.

Few published studies have documented steelhead trout spatial and temporal spawning characteristics (Needham and Taft 1934; Briggs 1953; Reingold 1965; Cederholm, in press). Briggs (1953) suggested that steelhead that returned to spawning streams earliest would tend to spawn farther upstream than those that returned later. Information on the spawning of sympatric summer and winter steelhead is notably lacking. In British Columbia, Smith (1969) found that summer steelhead migrated farther than winter steelhead prior to spawning and suggested possible spatial isolation. Everest (1973) speculated that spatial and temporal differences occurred between summer and winter steelhead spawners in an Oregon river. These studies infer that reproductive isolation mechanisms may be of importance in the maintenance of racial discreteness within summer and winter steelhead populations.

The Kalama River, a lower Columbia River tributary located in southwest Washington, contained sympatric indigenous summer and winter steelhead populations prior to the first annual introduction of summer steelhead from the Skamania Hatchery (Washougal River) in 1951, and winter steelhead from the Beaver Creek Hatchery (Elochoman–Cowlitz River stock) in 1955. It is unlikely that these introduced hatchery stocks were as genetically adapted to local Kalama River conditions as were the preexisting indigenous populations. The subsequent influence of naturally spawning stocks on the genetic characteristics of existing populations is unknown. Therefore, following Reisenbichler and McIntyre (1977) and Nicholas et al. (1978), we use the term "wild" to refer to those naturally produced steelhead having indigenous and/or hatchery stock lineages. In view of the current lack of evidence regarding the full extent of genetic differentiation between wild and hatchery steelhead stocks, we believe it is best to assume that wild stocks more closely represent locally adapted genetic resources than do hatchery stocks. This assumption forms the basis for comparison in the present study.

The presence of both hatchery and wild steelhead in the Kalama River system complicates the understanding of sympatric interactions between summer and winter steelhead races. Since all four groups of steelhead (wild and hatchery summer fish and wild and hatchery winter fish) return to and spawn naturally within the Kalama River watershed, the potential for extensive genetic exchange exists if spatial and/or temporal overlap in reproductive activity occurs. Our primary objective was to examine the temporal and spatial spawning characteristics among the four different groups of steelhead spawning naturally within the Kalama River watershed in order to evaluate (1) the extent of potential reproductive isolation between summer and winter races and (2) how this isolation might be influenced by hatchery steelhead.

Materials and Methods

Adult Tagging

We had access to upstream migrant adult summer and winter steelhead at the Washington Department of Fisheries Kalama Falls Salmon Hatchery (KFH). This facility is located approximately 17 km upstream from the mouth of the Kalama River. A partial anadromous barrier falls (3.4 m high) was circumvented by a fishway that terminated in a trap. Trapped steelhead were transferred to a nearby holding pond, anesthetized, and a numbered Floy anchor tag was inserted in each fish at the left posterior base of the dorsal fin. These externally visible tags were color-coded as to steelhead race (summer or winter-run) and origin (hatchery or wild) to allow identification of different spawner groups upon subsequent observation during the spawning period. The four tag colors used were orange and white for wild and hatchery winter steelhead, respectively, and green and yellow for wild and hatchery summer steelhead, respectively. Upon completion of tagging, fish were placed into a plastic pipe containing running water, which transported the fish back to the river where they were deposited into an open-ended, semicircular wire pen near the river bank. This enclosure minimized the potential for fish to be swept back downstream over the falls while still under the influence of anesthesia and also provided an area from which they could move upstream at will upon recovery. Not all adults moved upstream via the fishway. As determined through upper river snorkel surveys (similar to Northcote and Wilkie 1963), an average of 1.6 summer steelhead jumped over the falls for every summer steelhead observed through the fishway from 1976 to 1981. Therefore, not all steelhead spawners in the watershed received tags.

Field determinations of steelhead races for tagging purposes were similar to Smith (1969) and were based primarily on physical appearance and relative sexual maturity at the time of observation at KFH. In the spring, when races overlapped, summer steelhead exhibited typical marine coloration, having bright silver coloration along and below the lateral line. Gills were bright red and were free from parasitic copepods. Sexually mature winter fish possessed typical rainbow-like spawning coloration and marked sexual dimorphism. Gills of winter steelhead were pale red and had several to many copepods present. These characteristics were reversed in late fall and early winter, when races again overlapped. Winter steelhead were then the fresh-run fish. However, gonadal development in summer steelhead had not progressed to the same level observed in winter steelhead in the spring. Any steelhead for which confident racial determination could not be made were passed upstream untagged.

Dorsal fin deformity (stubbing) is commonly used by the Washington Department of Game and others as a tool to distinguish hatchery from wild steelhead in the field and is the method we employed. Fin erosion of this type, observed for a number of cultured salmonid species, is probably related to high fish rearing densities (Davis 1967; Novotny 1980; Moring 1982). Wild steelhead are normally not known to display deformed dorsal fins. The extent of visible dorsal stubbing existed along a continuum from barely perceptible to nearly total absence of the dorsal fin. We made a concerted effort to

standardize decision making in borderline instances of dorsal stubbing.

Spawning Surveys

Gobar Creek, a major Kalama River tributary 14 km above the tagging location, was used for observations of steelhead spawners and redds. The creek provides major steelhead spawning and rearing habitat and is characterized by moderate gradient, relatively abundant spawning substrate, and riparian vegetation. The total length of the creek is approximately 10 km, with an anadromous barrier falls at 8 km. Most adult hatchery summer steelhead returning to the Kalama River during our study had been conditioned in a rearing pond along Gobar Creek (4.7 km upstream from the mouth) for approximately 2 mo prior to liberation as smolts. Approximately 25% (26 000) of these smolts had been released directly into Gobar Creek at the pond outlet. The remaining summer steelhead smolts and all hatchery winter smolts had been trucked to traditional lower Kalama River mainstem sites for release. The pond was not used for hatchery winter steelhead rearing at any time prior to or during our study.

Spawner surveys conducted in Gobar Creek during 1976–77, 1977–78, and 1978–79 indicated that most steelhead spawning occurred from 1.5 km up to the barrier falls. In order to observe potential spatial segregation of spawner groups, the accessible length of the creek was divided into two nearly equidistant segments: the lower, from 1.6 km up to the pond water intake structure (length 3.3 km), and the upper, from the intake structure to the barrier falls (length 3.1 km).

Spawning surveys were conducted approximately once per week from December through May during 1979–80, 1980–81, and 1981–82. Intermittent surveys to observe actively spawning, tagged steelhead were also conducted in various stream sections from 1976–77 to 1981–82. Unless stated otherwise, data presented in this paper include results from simultaneously conducted, paired (upper and lower sections) surveys only. A spawning survey consisted of slowly walking upstream and enumerating the number of redds and number and tag colors of any tagged steelhead observed. When a redd was located, the site description was recorded and the redd was marked to avoid counting it on subsequent surveys.

Data Analysis

We examined the occurrence and magnitude of spawning activity for each of the four steelhead spawner groups by estimating the number of redds per spawner group using tag observation and redd count data. We emphasize that estimates of redds per spawner group should not be strictly interpreted as estimates of actual redds constructed by each of the four groups of spawners. The number of possible interactive combinations considering all spawner group and gender categories is obviously much greater than four. Instead, redds per spawner group reflect indices that indicate the potential relative reproductive activity (intensity) among the four spawner groups. A redd of one of the four spawner groups in this context would imply that at least one of the spawners involved in the spawning act was of this particular spawner group. Estimates of this type were required in order to estimate the relative proportional exchange in reproductive activity between spawner groups. Each survey season was divided into six monthly time intervals, from December through May. The following equation was used to estimate the number of redds ($\hat{R}$) per spawner group (i) for each monthly interval (j):

$$(1)\quad \hat{R}_{ij} = \frac{T_{ij} N_j}{T_j}$$

where T_{ij} is the number of tags observed for spawner group i during month j, N_j is the total number of new redds counted during month j, and T_j is the number of all tags observed during the month j.

In 1979–80, all steelhead passed upstream from the hatchery received tags. However, in 1980–81 and 1981–82, exceptionally large run sizes resulted in many hatchery summer fish and a few wild summer fish being passed upstream untagged. This was adjusted for by multiplying T_{ij} by the ratio of all fish per group to tagged fish per group that were passed upstream. Also, each year a number of fish of indeterminate race were passed upstream untagged. Because no racial identity could be established, these were assumed to consist of 50% summer steelhead and 50% winter steelhead, and therefore, no further adjustment of data was made.

For each spawning season, the average date of spawning for each spawner group ($\overline{DS}_i$) was estimated utilizing the following relationship:

$$(2)\quad \overline{DS}_i = \frac{\Sigma (\hat{R}_{ij})(D_j)}{R_i}$$

where $\hat{R}_{ij}$ is the estimated number of redds for spawner group i during month j, D_j is the number of consecutive days to the middle of month j (e.g. December = 15.5, January = 46.5, May = 166.5), and R_i is the total number of estimated redds for spawner group i.

Analysis of variance and Student–Newman–Keuls (SNK) tests (Zar 1974) were used to compare estimated spawning times among spawner groups over the 3-yr period. Estimates of $\hat{R}_i$ were standardized by section lengths (redds per kilometre) in respective upper and lower areas for comparisons of spatial differences (t-test). To determine whether the abundance of estimated hatchery summer redds in Gobar Creek was more likely due to (1) stream or area-specific homing tendencies (hatchery summer smolts released from and returning to Gobar pond) or to (2) random processes independent of release site, we employed a log-likelihood test (G statistic).

To estimate the extent of potential reproductive overlap among spawner groups, we estimated proportional spawning activity using redd estimate data for each spawning year. This procedure incorporated both spatial and temporal factors and resulted in an estimator of potential proportional interaction for each spawner group. Two primary functions were used to develop these estimates. The relationships estimated the proportion of total spawning activity for spawner group x associated with pure x by x matings ($P_{x,x}$) and mixed x by y matings ($P_{x,y}$). The equations were as follows:

$$(3)\quad P_{x,x} = \left[\frac{1}{\Sigma \hat{R}_{xj}}\right]\left[\Sigma \frac{(\hat{R}_{xj})^2}{N_j}\right]$$

$$(4)\quad P_{x,y} = \left[\frac{1}{\Sigma \hat{R}_{xj}}\right]\left[\frac{\hat{R}_{xj}\hat{R}_{yj}}{N_j}\right]$$

where $\hat{R}_{xj}$ and $\hat{R}_{yj}$ are the estimated numbers of redds for spawner groups x and y, respectively, during month j and N_j is as defined previously.

Finally, to account for potential spatial segregation, measures of proportional overlap were calculated for both upper and

TABLE 1. Number of upstream migrant adult steelhead observed at the Kalama Falls Salmon Hatchery for the 1979–80 through 1981–82 spawning seasons. Annual percentages in parentheses.

Spawning season	Summer steelhead		Winter steelhead		Unknown race[a]		Total
	Hatchery	Wild	Hatchery	Wild	Hatchery	Wild	
1979–80	1721 (43.3)	526 (13.2)	570 (14.3)	1033 (26.0)	93 (3.1)	32	3 975
1980–81	7016 (55.1)	2276 (19.7)	465 (3.7)	2234 (17.5)	432 (5.8)	308	12 731
1981–82	8018 (74.1)	824 (7.6)	307 (2.8)	972 (9.0)	403 (6.5)	303	10 827
Average[b]	(57.5)	(12.9)	(6.9)	(17.5)	(5.1)		

[a]Racially indeterminate when observed at KFH; these fish passed upstream untagged. Percentage for hatchery and wild fish combined.
[b]Unweighted.

TABLE 2. Number of tagged and untagged steelhead, active spawnings, and superimposed redds observed during Gobar Creek spawning surveys, 1979–80 through 1981–82.

Spawning season	No. of surveys	Steelhead observed		Active spawner aggregations[b]		No. of superimposed redds[c]
		Untagged	Tagged[a]	No.	Proportion of total redds	
1979–80	14	47	67	8 (1)[d]	0.003	9
1980–81	15	329	334	44 (5)	0.014	31
1981–82	16	160	97	28 (1)	0.005	6
				Mean =	0.007	

[a]All spawner groups combined.
[b]Actively spawning pairs or groups.
[c]Number of redds placed over previously existing, older redds.
[d]Number involving tagged fish only.

lower survey sections, which were subsequently combined in a weighted average of proportional reproductive overlap per spawner group for the entire creek (P_i). Parameter P_i may range from 0, when no overlap occurs, to 1.0, when overlap is total. The following relationship was used for both pure ($P_{x,x}$) and mixed ($P_{x,y}$) matings:

$$(5)\quad P_i = \frac{P_{il}(\Sigma \hat{R}_{ijl}) + P_{iu}(\Sigma \hat{R}_{iju})}{\Sigma \hat{R}_{ijl} + \Sigma \hat{R}_{iju}}$$

where P_{il} and P_{iu} are the proportions of total spawning activity for spawner group i (from equations (3) or (4)) in the lower and upper survey sections, respectively, and $\hat{R}_{ijl}$ and $\hat{R}_{iju}$ are the number of redds for spawner group i in month j in the lower and upper survey sections, respectively.

Results

Of all the steelhead observed at KFH each year (range 3975–12 731), steelhead of undetermined race comprised the smallest average percentage (5.1%), and hatchery summer steelhead the largest (57.5%). Relatively few hatchery winter steelhead were observed (Table 1). Tagged steelhead were observed in greatest numbers in 1980–81, whereas the fewest were found in 1979–80 (Table 2). More tagged than untagged steelhead were observed each year with the exception of 1981–82. Although actively spawning tagged steelhead were observed each year, this occurrence was quite low, averaging only 0.7% of all redds enumerated during paired surveys (Table 2). The steelhead redd density observed for the total spawning zone of Gobar Creek was 46.6, 54.8, and 32.2 redds·km^{-1} in 1979–80, 1980–81, and 1981–82, respectively. In all three years, a majority of total redds involved activity by hatchery summer steelhead (range 131.8–269.7 redds), and the lowest number of redds involved hatchery winter steelhead (range 1.9–8.0 redds) (Table 3).

Temporal Distribution

Estimates of redds per month for each spawner group in upper and lower survey sections were variable between years (Table 3). In general, however, hatchery summer fish spawned first, followed by wild summer, hatchery winter, and wild winter steelhead, respectively. Wild summer steelhead spawning was observed over the longest time period, from December through April. Spawning of the other three spawner groups was generally completed during a shorter period of time (<4 mo). Wild winter steelhead spawning occurred primarily from March to May.

We estimated the average date of spawning for each spawner group as January 10, February 15, March 6, and April 14 for hatchery summer, wild summer, hatchery winter, and wild

TABLE 3. Observations of steelhead groups (T) and redds (N), including the estimated number of redds per spawner group ($\hat{R}$), for Gobar Creek by month for the 1979–80, 1980–81, and 1981–82 spawning seasons. HS = hatchery summer; WS = wild summer; HW = hatchery winter; WW = wild winter steelhead.

Spawning season	Survey area	Month	T				N	$\hat{R}$			
			HS	WS	HW	WW		HS	WS	HW	WW
1979–80	Lower	Dec.	8	0	0	0	41	41.0	0	0	0
		Jan.	14	0	0	0	43	43.0	0	0	0
		Feb.	2	0	0	0	25	25.0	0	0	0
		Mar.	0	0	0	1	14	0	0	0	14.0
		Apr.	0	0	1	10	30	0	0	2.7	27.3
		May	0	0	0	2	11	0	0	0	11.0
	Upper	Dec.	11	1	0	0	55	50.4	4.6	0	0
		Jan.	10	0	0	0	48	48.0	0	0	0
		Feb.	2	1	0	0	6	4.0	2.0	0	0
		Mar.	0	1	0	0	16	0	16.0	0	0
		Apr.	0	0	0	3	3	0	0	0	3.0
		May	0	0	0	0	6	0	0	0	(6.0)[a]
	Totals (%)						298	211.4 (70.9)	22.6 (7.6)	2.7 (0.9)	61.3 (20.6)
1980–81[b]	Lower	Dec.	150	1	0	0	20	19.9	0.1	0	0
		Jan.	153	20	1	0	104	91.4	12.0	0.6	0
		Feb.	20	0	0	0	23	23.0	0	0	0
		Mar.	0	0	0	2	16	0	0	0	16.0
		Apr.	0	0	0	3	14	0	0	0	14.0
		May	0	0	0	0	7	0	0	0	(7.0)[a]
	Upper	Dec.	29	2	0	0	8	7.5	0.5	0	0
		Jan.	71	8	1	1	103	90.2	10.2	1.3	1.3
		Feb.	10	1	0	0	36	32.7	3.3	0	0
		Mar.	(5)[c]	(1)[c]	0	0	6	5.0	1.0	0	0
		Apr.	0	1	0	0	14	0	14.0	0	0
		May	0	0	0	0	0	0	0	0	0
	Totals (%)						351	269.7 (76.8)	41.1 (11.7)	1.9 (0.5)	38.3 (10.9)
1981–82[d]	Lower	Dec.	60	1	0	0	50	49.2	0.8	0	0
		Jan.	12	1	0	0	25	23.1	1.9	0	0
		Feb.	0	2	0	0	26	0	26.0	0	0
		Mar.	0	0	1	0	8	0	0	8.0	0
		Apr.	0	0	0	5	10	0	0	0	10.0
		May	0	0	0	0	5	0	0	0	(5.0)[a]
	Upper	Dec.	46	11	0	0	26	21.0	5.0	0	0
		Jan.	18	2	0	0	26	23.4	2.6	0	0
		Feb.	4	0	0	0	10	10.0	0	0	0
		Mar.	(2)[c]	0	0	(1.5)[c]	9	5.1	0	0	3.9
		Apr.	0	0	0	3	7	0	0	0	7.0
		May	0	0	0	0	4	0	0	0	(4.0)[a]
	Totals (%)						206	131.8 (64.0)	36.3 (17.6)	8.0 (1.8)	29.9 (14.5)

[a]No tagged steelhead were observed; however, because only WW steelhead had been observed for the previous month and no steelhead were observed the following month, redds were assumed to have been made by WW spawners.

[b]HS observations were multiplied by 1.46 and WS by 1.24 to expand for fish passed upstream at KFH without tags (expansion factor = total tagged + untagged steelhead/tagged steelhead).

[c]No tagged steelhead were observed; however, since redds were observed, it was necessary to estimate spawner composition. Values were estimated by averaging numbers of tags from the months immediately preceding and immediately following.

[d]HS observations were multiplied by 1.97 to expand for steelhead passed upstream at KFH untagged.

winter steelhead, respectively (Table 4). Average time of spawning was significantly different among spawner groups ($P < 0.01$; $F_{3,8} = 7.82$). Subsequent SNK testing revealed significant differences ($P < 0.05$) in timing between hatchery summer and wild winter steelhead and between wild summer and wild winter steelhead. No significant differences were found between hatchery winter and wild winter steelhead, nor between hatchery winter and hatchery summer steelhead. This ambiguity necessarily precluded statistical testing of hatchery versus wild summer steelhead and wild summer versus hatchery

TABLE 4. Temporal spawning data for Gobar Creek steelhead, 1979–80 through 1981–82. Data converted to calendar dates following calculation of $\overline{DS}_i$.

Spawner group	Mean date of spawning			Grand mean[a]	SD
	1979–80	1980–81	1981–82		
Hatchery summer	Jan. 7	Jan. 20	Jan. 6	Jan. 10	8.6
Wild summer	Feb. 23	Feb. 21	Feb. 1	Feb. 15	12.2
Hatchery winter	Apr. 15	Jan. 16	Mar. 16	Mar. 6	45.5
Wild winter	Apr. 16	Apr. 5	Apr. 20	Apr. 14	8.0

[a]Unweighted mean of 1979–80 through 1981–82.

winter steelhead. These problems appeared to be due primarily to the scarcity of observations of hatchery winter fish. If hatchery winter steelhead had not been present, then it seems probable that differences would have been significant between the three remaining spawner groups.

Occasionally, redds were observed either partially or completely on the same specific site as preexisting redds. These superimposed redds were enumerated when site overlap was thought to be total (Table 2). Though not rigorously quantitative due to subjectivity in determination of "total" overlap, our data do confirm the occurrence of this phenomenon in Gobar Creek.

Spatial Distribution

Both the lower and upper survey sections were used for spawning by all spawner groups. Hatchery and wild summer steelhead spawning activity was distributed randomly between survey sections ($P \geq 0.05$). Although not statistically significant overall, the number of redds per kilometre for wild winter steelhead was greater in the lower section than in the upper section in two of the three spawning seasons. Data for hatchery winter steelhead were inconclusive due to inadequate sample sizes.

The proportion of hatchery summer steelhead within the entire Kalama watershed was not significantly different from the proportion of estimated hatchery summer steelhead redds in Gobar Creek ($P \geq 0.05$). Therefore, the high abundance of hatchery summer redds in Gobar Creek was the result of influences other than release or rearing site (i.e. random homing by large hatchery summer steelhead run sizes), and was most likely due to influences common throughout the Kalama drainage.

Reproductive Interaction

During spawning surveys, actively spawning steelhead pairs or aggregations were often encountered (Table 2). Few of these active redds were occupied only by tagged steelhead. Most involved at least one untagged fish. However, when all of the participants were tagged, it was possible to observe directly the occurrence of interracial and intraracial mating. Of 14 total tagged spawner aggregations observed from 1977–78 to 1981–82 (intermittent surveys included), eight were intragroup, hatchery summer × hatchery summer crosses. Two of these, however, were accompanied by a generally smaller, subdominant wild summer satellite male. Hatchery summer × wild summer crosses and wild winter × wild winter crosses were each observed twice. One wild summer × wild winter cross was observed. Two other potential examples of interracial mating occurred when a hatchery winter and a wild winter satellite male were each observed with hatchery summer × hatchery summer pairs. Whereas these data do not allow direct inferences on the magnitude of reproductive exchange among spawner groups, they do serve as prima facie evidence of interbreeding among the spawner groups under natural conditions.

Potential reproductive exchange, or overlap (P_i), among spawner groups was found to be highly variable between years (Fig. 1). In general, overlap was greatest in 1980–81 and least in 1979–80. In 1980–81, virtually all possible spawner group comparisons resulted in some degree of overlap, whereas in the remaining years, only half of the possible pairings resulted in overlap.

Based on an average of the 3-yr of data, most reproductive activity involved interactions among members of the same spawner group (Table 5). Wild winter and hatchery summer steelhead were the most reproductively discrete spawner groups, having 95.1 and 93.0% intragroup spawning activity, respectively. Reproductive exchange was greatest for hatchery winter steelhead, with only 36.7% intragroup crosses, and intermediate for wild summer steelhead, which had 63.9% intragroup crosses.

The extent of intergroup reproductive exchange ranged from 35.8% for the contribution of hatchery summer fish to all matings involving wild summer steelhead to 0.1% for the contribution of wild summer steelhead to all matings of wild winter steelhead (Table 5). Interracial matings generally occurred to a lesser extent than intraracial matings.

Discussion

We based estimates of spawning activity for each spawner group on the assumption that the incidence of dorsal fin deformity was a valid technique to distinguish hatchery from wild steelhead in the field. Although extensive controlled comparative studies are lacking, fin deformity information is available for hatchery summer steelhead returning to the Kalama River system. In each of two consecutive summer-run return years the entire dominant two-salt hatchery origin age group returned with right ventral or left ventral fin marks, allowing positive identification. From observations of adults at KFH, the proportion of fin-marked fish that were unstubbed varied from 1.4% in 1978 to 8.1% in 1979. No marking studies have been conducted to evaluate the incidence of natural dorsal deformity for wild steelhead. However, analysis of scales from wild summer and wild winter adults (≥2 yr of freshwater growth) returning to the Kalama River during our study indicated that an average of 7.6 and 3.8% had deformed dorsals, respectively. We feel that the high incidence of dorsal deformity in known hatchery summer steelhead and the low incidence of

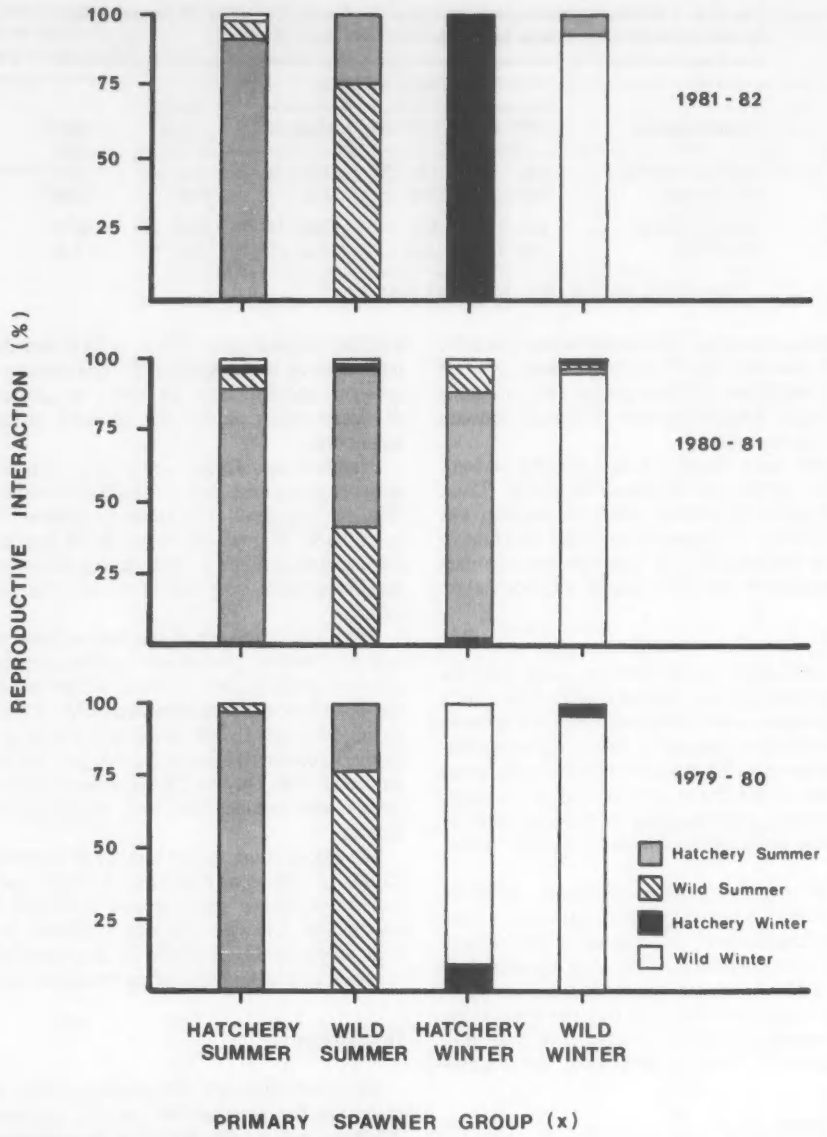

FIG. 1. Relative proportional reproductive interaction (P_i) among hatchery summer, wild summer, hatchery winter, and wild winter spawner groups, 1979–80 to 1981–82.

naturally stubbed wild steelhead returning to the Kalama River in these years supports the use of dorsal stubbing as we described.

Observations of differentially tagged steelhead in the act of spawning provide the best evidence of reproductive interaction among spawner groups, but the number of these observations was limited. Therefore, we used the redds per spawner group data base as our main source for comparison. This approach assumes that mate selection among steelhead spawner groups was random. In Pacific salmon (*Oncorhynchus* spp.), species recognition and mate selection among spawners has been shown to have a visual and olfactory basis, the specific mechanism apparently dependent upon the sex of individual spawners (Duker 1981). Duker (1981) found that salmon were successful in locating and eventually spawning with conspecifics. However, it is unknown whether more refined race-specific or wild-specific mate selection occurs. If group-specific mate selection exists among sympatric steelhead, then our analyses would overestimate the amount of genetic interaction between spawner groups.

While reproductive overlap occurred at low levels in both 1979–80 and 1981–82 relative to 1980–81, it is interesting to note that the 1980–81 spawning season was the first following the May 18, 1980, volcanic eruption of nearby Mt. St. Helens. Physical and biological impacts on the Columbia River and tributaries downstream from the volcano were extensive (Whit-

TABLE 5. Average proportional reproductive interaction (P_i) among Gobar Creek spawner groups, 1979–80 to 1981–82. Intraracial totals in parentheses.

Primary spawner group (x)	Interactive spawner group (y)			
	Summer steelhead		Winter steelhead	
	Hatchery	Wild	Hatchery	Wild
Hatchery summer	0.930 (0.991)	0.061	0.002 (0.009)	0.007
Wild summer	0.358 (0.997)	0.639	0.002 (0.003)	0.001
Hatchery winter	0.292 (0.327)	0.035	0.367 (0.673)	0.306
Wild winter	0.035 (0.036)	0.001	0.013 (0.964)	0.951

man et al. 1982). The eruption occurred during the very early stages of the 1980 summer steelhead upstream migration, and it is possible that "normal" adult migrations of adult steelhead through affected Columbia River areas were altered to some extent. Additionally, and more importantly, it is possible that straying rates into the Kalama from nearby affected river systems were abnormally high, resulting in an unusually large infusion of non-Kalama steelhead. Therefore, the increased level of reproductive exchange calculated for Gobar Creek in 1980–81 may have been at least partly related to this relatively rare environmental perturbation. Average reproductive overlap may normally be lower than indicated by our results.

Spawning by each of the four spawner groups occurred in both upper and lower stream survey sections. Winter steelhead displayed a slight tendency to spawn in the lower part of Gobar Creek. This observation weakly confirmed the findings of Briggs (1953), who suggested that steelhead that returned later would tend to spawn downstream from those that returned earlier. Summer steelhead spawner groups, however, did not conform to this hypothesis. Although they were the first groups to enter the Kalama River, they spawned in Gobar Creek on a spatially random basis. For hatchery summer steelhead, possibly this was partly because a rearing pond was located along the lower survey section. Precise homing by hatchery steelhead to a release site or area has been documented (Wagner 1969; Cramer 1981). Therefore, because the pond was used exclusively for summer steelhead rearing, adult homing to the area of rearing or release could have altered a natural tendency of this group to continue migrating up Gobar Creek. The presence of tagged hatchery summer steelhead prespawners in pools near the pond outlet in the fall of each year supports this interpretation. Our data, therefore, are inconclusive regarding spatial isolation for all groups.

Numerous studies indicate that salmonid stocks can be altered behaviorally and genetically through the domestication process, both intentionally (Donaldson 1970; Ricker 1972; Gall and Gross 1978; McIntyre and Amend 1978) and unintentionally (Greene 1952; Vincent 1960; Møller 1970; Kincaid 1976; Allendorf and Phelps 1980). For example, the time of sexual maturation has a hereditary component for Skamania summer steelhead (Ayerst 1977) and Alsea winter steelhead (Rosentreter 1977). In each case, hatchery selection for early spawning steelhead advanced maturation time after only a few generations. On average, we found that summer and winter hatchery fish in Gobar Creek spawned approximately 1 mo prior to their wild counterparts. Apparently, hatchery steelhead spawning in the wild environment largely maintain the time of maturity found in the hatchery environment. This temporal segregation reduces the potential for genetic exchange between hatchery and wild stocks in nature.

Our results indicate that summer and winter steelhead in Gobar Creek are not completely reproductively isolated. Although not without alternative explanations (i.e. misidentification, or straying effect), we feel that our direct observations of interracial spawning and the occurrence of reproductive overlap in spatial and temporal spawner distribution strongly suggest that gene flow among summer and winter steelhead does take place. However, estimated reproductive overlap between wild summer and wild winter steelhead was very low. Since we feel that wild steelhead genetically most closely resemble indigenous populations, such isolation was not totally unexpected. This finding conflicts with Chilcote et al. (1980), who found no evidence of reproductive isolation between wild summer and winter steelhead in the Kalama River on the basis of electrophoretic analyses. The absence of electrophoretic differentiation between races as described by Chilcote et al. (1980), however, does not necessarily preclude the existence of genetic differences (Utter 1981). Based on Smith (1969), who found that interracial genetic exchange in steelhead was absent or at least very rare on the basis of taxonomic and physiological characteristics, and the results of our study, we conclude that some degree of successful reproductive isolation (restriction of gene flow) in sympatric wild steelhead does occur, thereby providing a mechanism for the maintenance of racial identity.

Complete reproductive isolation was not observed between hatchery and wild steelhead in Gobar Creek. The potential gene flow from hatchery to wild winter steelhead was minimal due to the scarce and relatively variable temporal nature of hatchery winter steelhead. A preponderance of hatchery summer spawners and their temporal proximity to wild summer spawners resulted in the potential for substantial gene flow from hatchery to wild summer steelhead. Apparently, spatial and temporal spawner segregation was of insufficient magnitude to prevent considerable interbreeding between these two spawner groups. In general, given spatial and temporal spawning characteristics similar to what we observed, the larger a population of hatchery spawners is relative to the size of the wild population, the greater the opportunity for hatchery introgression into the wild population.

The adaptive value of small amounts of reproductive exchange among sympatric races of wild steelhead may be to provide local populations with a mechanism to respond to environmental changes by utilizing new genetic variability. In this context, gene flow would act to allow populations to expand and adjust to new, diverse niches. It is also possible, however, that the apparent high degree of intermixing between wild and non-synchronous spawning domesticated steelhead, if successful in the production of viable offspring, could contribute to alter the state of reproductive isolation between sympatric wild steelhead populations in the Kalama River over time, resulting in potential reduction of locally adapted wild stock traits.

Steelhead of various groups were found to be spawning in Gobar Creek continuously from December through May, and it is likely that spawning by wild winter steelhead could even have extended into the month of June. Since environmental conditions are generally highly variable over this length of time

(≥6 mo), a question exists regarding the relative survival of eggs and alevins from early and late redds. Redds constructed in the late fall and early winter months are generally adversely subjected to considerable scouring and relatively high turbidity conditions (Cederholm, in press). Additionally, redd superimposition by later spawning steelhead occurs (Needham and Taft 1934; Reingold 1965; Orcutt et al. 1968; this study), which may result in a negative impact to preexisting redds through disturbance and associated mortality of incubating embryos and alevins (Orcutt et al. 1968). These factors, in conjunction with the relative temporal differences observed, would appear to favor a greater reproductive efficiency for wild winter steelhead than for other groups.

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Relationships between the Partitioning of Trace Metals in Sediments and Their Accumulation in the Tissues of the Freshwater Mollusc *Elliptio complanata* in a Mining Area

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Surficial sediment samples and specimens of a benthic freshwater pelecypod, *Elliptio complanata*, were collected at eight sites located along a metal contamination gradient downstream from the mining and smelting complex in Rouyn–Noranda, Quebec. The molluscs were dissected, digested, and analysed for Cu, Pb, Zn, Fe, and Mn by flame atomic absorption spectrophotometry. Each sediment sample was wet sieved and the <70-μm granulometric fraction was subjected to a sequential extraction procedure designed to determine the partitioning of the trace metals among various geochemical phases. Regression analysis indicates that Cu, Pb, and Zn levels in various tissues of *E. complanata* (or in the whole organism, without the shell) are best related, not to total metal concentrations in the adjacent sediment, but rather to one or more of the relatively easily extracted fractions. Accumulation of Cu, Pb, and Zn was also influenced by the protective or competitive effect of other sediment constituents, notably amorphous iron oxyhydroxides and, to a lesser degree, organic matter.

Des échantillons de sédiments de surface et des spécimens du pélécypode d'eau douce *Elliptio complanata* ont été prélevés à huit sites situés dans un gradient de contamination en métaux, en aval du complexe d'opérations minières et de raffinage de Rouyn–Noranda, Québec. Les mollusques ont été disséqués, digérés et analysés pour Cu, Pb, Zn, Fe et Mn par spectrophotométrie d'absorption atomique. Chaque échantillon de sédiments était tamisé par voie humide et la fraction granulométrique <70 μm était soumise à une procédure d'extractions séquentielles conçue pour déterminer la répartition de métaux traces entre diverses phases géochimiques. L'analyse de régressions indique que les niveaux de Cu, Pb et Zn dans divers tissus de *E. complanata* (ou dans l'organisme entier, sans la coquille) sont mieux corrélés avec l'une ou plusieurs des fractions de métaux extraites relativement facilement des sédiments plutôt qu'avec les concentrations totales des métaux dans ces sédiments. L'accumulation de Cu, Pb et Zn était aussi influencée par l'effet protecteur ou compétitif d'autres constituants des sédiments, notamment des oxyhydroxydes de fer amorphe et, à un degré moindre, de la matière organique.

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The tendency of many aquatic organisms to accumulate trace metals from their environment has led to the use of some of these organisms for the assessment of trace metal pollution (Phillips 1977; Wright 1978). Bivalve molluscs have been used extensively for quantitative assessment of trace metal contamination, especially in the marine environment (Huggett et al. 1973; Darracott and Watling 1975; Phillips 1976; Klumpp and Burdon-Jones 1982; Ritz et al. 1982). The use of freshwater pelecypods as biological indicators has also been suggested (Curry 1977; Foster and Bates 1978; Jones and Walker 1979; Forester 1980). Direct proportionality between environmental concentrations and tissue levels is frequently assumed in such studies. However, the processes and factors controlling trace metal availability to these organisms are not completely understood, and simple relationships are seldom found in natural systems between trace metal levels in the organisms and total metal concentrations in either the sediment or the water to which the organisms are exposed.

The objective of this study was to identify factors influencing trace metal bioavailability to freshwater benthic organisms; to this end, we have investigated the relationships between the partitioning of trace metals in sediments and their accumulation in various tissues of the benthic pelecypod *Elliptio complanata*. This filter feeder is widely distributed in the streams and lakes of northeastern North America (Clarke 1973; Curry 1977; Heit et al. 1980; Magnin and Stanczykowska 1970); it is a long-lived sedentary organism that comes into contact with both sediments and water when feeding and respiring, and can accumulate trace metals. These characteristics make *E. complanata* a useful organism for studying the factors influencing trace metal bioavailability in the aquatic environment.

Laboratory experiments have shown that the ingestion of sediments can be an important route of trace metal uptake for some marine bivalves and that the uptake should depend upon the physicochemical associations of the trace metals in the sediments (Jenne and Luoma 1977; Luoma and Jenne 1977). An alternative route of entry involves the direct absorption of trace metals from water (Jenne and Luoma 1977; Luoma and Jenne 1977; George and Coombs 1977; Simkiss et al. 1982; Zamuda and Sunda 1982). Recent studies (Oakley et al. 1981; Luoma

and Davis 1983) suggest that adsorption onto specific geochemical phases might control the trace metal concentrations in the ambient water at the sediment–water interface (i.e. the interstitial water or the water in intimate contact with the surface of the sediments). The physicochemical forms of trace metals in the sediments could thus affect the bioavailability of particulate trace metals ingested by filter-feeding organisms, and also reflect the dissolved trace metal concentrations in the solution to which these organisms are exposed. If these observations may be generalized for natural systems, there should be relationships between the trace metal levels in specific tissues (or in the whole organisms) and the concentrations of certain metal forms in the sediments.

Study Area

Necessary conditions for the present study included the existence of a spatial gradient of trace metal concentrations in the sediments and the presence of an appreciable bivalve population along this gradient. Such conditions were met in the mining area of Rouyn–Noranda, located in northwestern Quebec some 500 km northwest of Montreal, for stations chosen in Lakes Beauchastel, La Bruère, and Montbeillard. Sources of metal contamination in the area include abandoned mines, current mining operations, accumulated residues from the smelting process, and atmospheric fallout from the smelter emissions. Lakes Beauchastel and La Bruère are influenced by point sources of metal contamination, while Lake Montbeillard is affected only by the diffuse smelter emissions.

The region is densely forested and has a low relief (<80 m); drainage is not well developed, and swampy areas, densely covered with vegetation, are abundant. The drainage network is part of the Ottawa River watershed. The bedrock is covered by locally derived till and, at the surface, by lacustrine clays deposited during the existence of the proglacial Lake Barlow–Ojibway; the mean depth of unconsolidated material is 15–20 m. The lacustrine clay fraction (<2 μm) contains the following minerals: illite > vermiculite > chlorite > feldspar > quartz > hornblende. Many metal anomalies are observed in the lacustrine clays (Lalonde and Lasalle 1983) and, in particular, high Cu, Pb, and Zn concentrations are reported for some soil samples. Metal concentrations found in the sediments may thus be considered to have been introduced both by anthropogenic activities and by natural processes of weathering and erosion.

Methods

During a preliminary sampling effort, in May 1981, surficial sediments were collected with an Ekman dredge at 46 sites in the lakes lying downstream from the major mining and smelting activities in Rouyn–Noranda. A diver equipped with SCUBA noted the presence or absence of an appreciable mollusc population at each site. To evaluate the spatial variability of metal levels in the sediments, total concentrations of Cu, Pb, and Zn were determined for each of these samples. A second sampling campaign was then carried out between 3 and 5 September 1981 at eight sites (Fig. 1) located along the spatial gradient identified earlier. At each site, bottom sediments were obtained with an Ekman dredge; the surficial, oxidized portion was collected with a plastic spoon, placed in a polyethylene bag, and kept at 4°C until analysed; analysis was started within 4 d following the sampling. Specimens of *E. complanata* were obtained by a diver at each site, within a radius of 20 m from the sediment collection point. The specimens were cleaned by brushing, kept at 4°C during the field work and transport to the laboratory, and then frozen until needed for analysis.

All bivalves analysed were between 6.5 and 9.5 cm long, and all but one specimen were between 9 and 13 yr old. Specimens were dissected while still frozen (Smith et al. 1975) into foot, muscle, visceral mass, hepatopancreas, gills, and mantle. Each organ was dried (105°C), weighed, and individually digested and analysed for Cu, Pb, Zn, Fe, and Mn. The soft parts of the organisms remaining after dissection, comprising mainly the intestinal tract contaminated with sediments, were discarded; this material represented about 10% of the total soft parts (dry weight). For digestion of the organs, the following steps were employed (Borg et al. 1981): treatment with 10 mL of HNO_3 (70%) overnight at room temperature, evaporation to near dryness, and treatment with 10 mL of H_2O_2 (30%) heated to 85°C for 6 h. Nitric acid (final concentration 2%) was then added and the resulting solution analysed by flame atomic absorption spectrophotometry. Mean concentrations, C_R, for the whole organisms were calculated as follows for each trace metal:

$$(1) \quad C_R = \Sigma C_i W_i / \Sigma W_i$$

where C_i and W_i represent the trace metal level and the dry weight of the ith organ or tissue, respectively. The values of C_R could not be calculated for Pb, as Pb levels in some tissues were below the analytical detection limit. To estimate the size of particles ingested by *E. complanata*, the contents of the digestive system were obtained from five specimens from different sampling points and combined. The accompanying mucus was then destroyed with H_2O_2 (30%), and the distribution of particles was determined on a volumetric basis (Fig. 2) with an electronic particle counter (Coulter counter, model TA).

The sediment samples were wet sieved with a 70-μm Nylon screen, and the <70-μm granulometric fraction, assumed to be representative of that ingested by *E. complanata* (see Fig. 2), was retained. In the size separation, minimum amounts of water were used to avoid excessive solubilization of solid material; during the sieving of a given sample, the water was clarified by high-speed centrifugation and reused. The <70-μm granulometric fraction was subjected to a sequential extraction procedure designed to partition the particulate trace metals, M, into the following five fractions: (a) M(F1): *exchangeable metals*. The sediment sample was extracted for 10 min with 0.5 mol $MgCl_2$/L at pH 7.0; (b) M(F2): *metals bound to carbonates or specifically adsorbed*. The residue from (a) was leached for 5 h with 1 mol sodium acetate (NaOAc)/L adjusted to pH 5.0 with acetic acid (HOAc); (c) M(F3): *metals bound to Fe–Mn oxides*. The residue from (b) was extracted at 96°C for 6 h with 0.04 mol $NH_2OH \cdot HCl$/L in 25% (v/v) HOAc; (d) M(F4): *metals bound to organic matter and sulfides*. The residue from (c) was extracted at 85°C for 5 h with 30% H_2O_2 adjusted to pH 2 with HNO_3 and then at room temperature with 3.2 mol NH_4OAc/L in 20% (v/v) HNO_3; (e) M(F5): *residual metals*. The residue from (d) was digested with a 5:1 mixture of concentrated hydrofluoric and perchloric acids.

The detailed experimental procedure is given in Tessier et al. (1979, 1980). Trace metal concentrations in the leachates were determined by flame atomic absorption spectrophotometry. Quantification was achieved with appropriate calibration curves prepared with the components of each extraction solution (sediments) and the digestion solution (mollusc tissues).

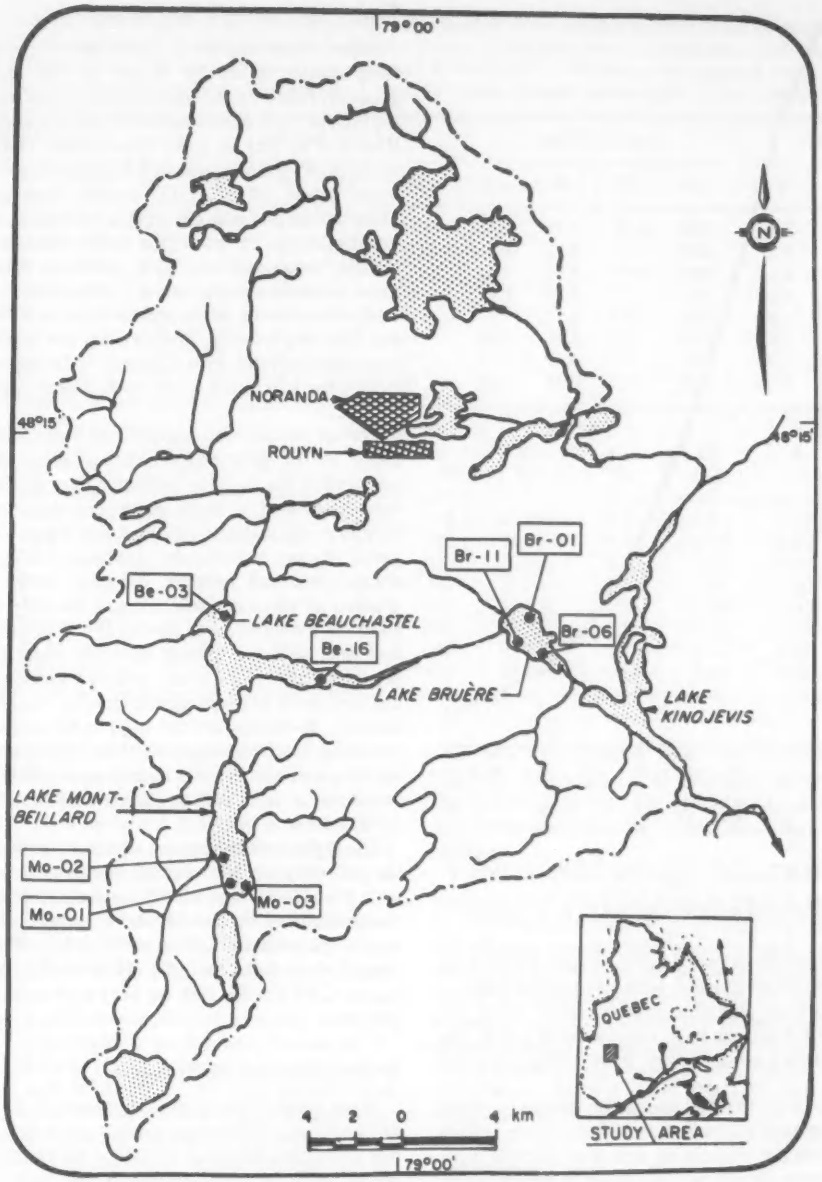

FIG. 1. Sampling stations in the Rouyn–Noranda area, Quebec.

Particulate sulfur and carbon were determined with Leco Total Sulfur (model IR-32) and Carbon (model IR-12) analyzers, respectively. Inorganic carbon was assumed to be equal to the difference between total and organic carbon concentrations, the latter being measured after acid treatment (6 mol HCl/L, 30 min) of the sediment to dissolve the carbonates present. Silicon and Al concentrations were determined by atomic absorption spectrophotometry (nitrous oxide–acetylene flame), after fusion with lithium metaborate followed by dissolution of the fused material in 1.2 mol HCl/L.

Results

Trace Metal Distribution in the Sediments (<70 μm Granulometric Fraction)

Total trace metal concentrations, M(S5), were consistently higher in sediments from Lakes Beauchastel and La Bruère than in those from Lake Montbeillard; the same trend was generally observed for the concentrations in the individual fractions 1–5 (Table 1). For total trace metal values, the ratios of maximum to

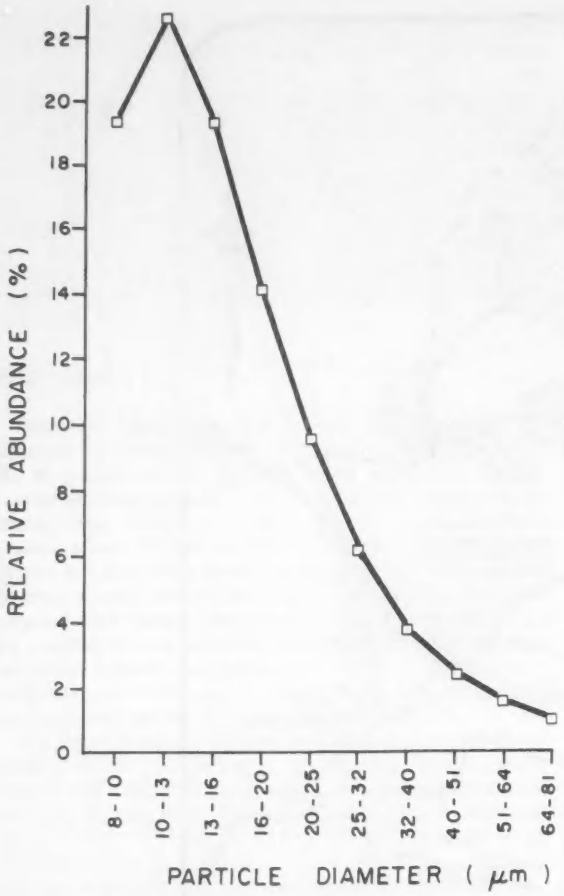

FIG. 2. Size distribution of particles ingested by *E. complanata*.

minimum concentration lay between 5 (Pb, Zn) and 8 (Cu); total Fe concentrations, on the other hand, were less variable (ratio < 2). A somewhat greater variation was observed when the individual nondetrital fractions were considered (F1–F4), for which the corresponding ratios ranged from 5 (Pb(F3)) to 34 (Zn(F1)).

The distribution of a given metal among the various fractions obtained by the sequential extraction procedure (Table 1) does not necessarily reflect the scavenging action of discrete sediment phases, but rather should be considered as operationally defined by the methods of extraction (Tessier et al. 1979). As has been observed in previous studies (Forstner and Wittmann 1981; Tessier et al. 1980, 1982), each metal exhibited a distinctive partitioning pattern among the nondetrital fractions 1–4. For example, the distribution of Cu showed the characteristic predominance of fraction 4, approximately equal contributions from fractions 2 and 3, and virtually undetectable levels in fraction 1 (i.e. F4 > F3 ~ F2 ≫ F1). In the case of Pb, fraction 3 dominated the distribution, the contributions from fractions 2 and 4 being approximately equal and that from fraction 1 again being quantitatively negligible (i.e. F3 > F2 ≥ F4 ≫ F1). A similar distribution pattern was observed for Zn, except that its exchangeable fraction contributed appreciably to the total metal load (i.e. F3 > F2 ≥ F4 ≥ F1).

Trace Metal Levels in the Tissues

Metal concentrations in the tissues of *E. complanata* varied from station to station (Table 2); the lowest levels were generally found for the sites located in Lake Montbeillard, while the highest were observed for the sites located in either Lake La Bruère (Cu, Pb) or Lake Beauchastel (Zn). A hierarchical analysis of variance indicated that significant differences in the tissue levels ($P < 0.05$) existed between sites in Lake Montbeillard and sites BR-01 (Cu in the gills, muscle, and in the total organism; Pb in the gills and mantle) or BE-03 (Zn in the muscle, hepatopancreas, gills, and in the total organism). The mean bioconcentration ratios (concentration in the molluscs/total concentration in the sediment) were 0.38 and 1.11 for Cu and Zn, respectively; these values are much lower than the concentration ratios of 4 (Cu) and 7 (Zn) reported by Heit et al. (1980) for specimens of *E. complanata* collected from Lake George.

For all metals, the highest concentrations were generally found in the gills and mantle, whereas the lowest values occurred in the foot and adductor muscle; intermediate levels were associated with the hepatopancreas and visceral mass. However, the standard deviations in Table 2 are high, and at a given station statistically significant differences were not always observed between different tissues. A hierarchical analysis of variance indicated that the only significant differences between the tissue levels ($P < 0.05$) at most stations are foot and adductor muscle different from gills, mantle, and hepatopancreas (for Cu) and adductor muscle and visceral mass different from gills and mantle (for Zn, Fe, and Mn). The large standard deviations are too high to be attributed to analytical error only (±8%), suggesting that biological variability (age, sexual condition, etc.) is largely responsible for the observed variations at each station (Boyden 1974, 1977; Cossa et al. 1979; Julshamn 1981).

The highest contributions to the metal body burden were from the gills (Cu, Zn, Mn) and the mantle (Fe) whereas the lowest were from the foot and adductor muscle (Table 3). Similar tissue distributions of Fe and Mn have been reported for the same species (Hobden 1970; Seah and Hobden 1969); especially high contributions from the gills (20% for Fe; 78% for Mn) and mantle (27% for Fe; 16% for Mn) were also observed by these authors.

Bivalve–Sediment Relationships

Trace metal levels in the soft tissues of *E. complanata* were first compared with the trace metal concentrations in each of the five extraction fractions ([M(F1)] to [M(F5)]) and with the partial sums of these concentrations ([M(S2)] to [M(S5)]). Multiple regressions were then performed with the trace metal concentration in the tissues as the dependent variable and the concentrations of metal in the various sediment fractions as the independent variables. Due to the high multicolinearity existing between the sediment fractions, the selection of the first and largest partial regression coefficient explained a large proportion of the variance, with little gained (2–3%) upon inclusion of further additive combinations of the fractions. Such inclusion also resulted in partial regression coefficients that were highly variable. Finally, the concentrations in each sediment fraction or combination were normalized with respect to the iron oxide or organic carbon content of the sediments (i.e. [M(F1)]/[Fe(F3)] to [M(S5)]/C_{org}), and bivariate analyses between the normalized parameters and tissue concentrations were

TABLE 1. Physicochemical characteristics of the <70-μm granulometric fraction of the sediment samples: trace metal concentrations as determined with the sequential extraction procedure; S, Al, Si, inorganic carbon, and organic carbon concentrations. All the results are based on sediment dry weight; [M(F1)]–[M(F5)] represent metal concentrations in fractions 1–5 following the sequence given in the text; [M(S5)] represents the sum of metal concentrations for the five fractions; C_O and C_I represent organic and inorganic carbon concentrations, respectively.

	[M(F1)] (μg/g)				[M(F2)] (μg/g)				[M(F3)] (μg/g)				[M(F4)] (μg/g)			
Site	Cu	Pb	Zn	Fe	Cu	Pb	Zn	Fe	Cu	Pb	Zn	Fe	Cu	Pb	Zn	Fe
Mo-01	0.1	<0.9	1.5	0.9	2.1	4.3	5.1	67.0	4.3	14.5	31.8	2830	5.2	<2.0	4.0	110
Mo-02	0.1	<0.9	1.1	2.6	1.9	2.6	7.2	90.7	6.0	20.8	51.5	4980	7.7	2.0	6.9	205
Mo-03	<0.1	<0.9	0.4	4.3	2.8	2.6	7.8	57.0	6.4	14.4	44.8	3880	4.8	<2.0	5.9	115
Be-03	<0.1	<0.9	19.1	7.0	17.5	8.6	64.5	670	13.8	26.8	177	7220	40.3	6.3	15.2	240
Be-16	<0.1	<0.9	16.0	2.7	13.7	14.9	65.5	280	7.9	27.0	206	5610	69.1	10.5	40.2	140
Br-01	0.2	<0.9	7.8	0.9	18.1	13.0	17.5	144	20.2	49.5	123	4950	28.7	8.3	13.3	920
Br-06	0.3	0.9	13.8	2.6	23.7	12.2	41.4	190	17.8	51.6	163	5160	54.9	8.4	18.6	195
Br-11	0.1	<0.9	13.4	2.7	27.8	16.7	49.1	185	31.4	77.0	215	5820	61.5	21.0	33.0	290

	[M(F5)] (μg/g)				[M(S5)] (μg/g)				C_O	C_I	S	Al	Si
Site	Cu	Pb	Zn	Fe	Cu	Pb	Zn	Fe	(%)	(%)	(%)	(%)	(%)
Mo-01	12.0	10.0	36.0	21 300	23.7	28.8	78.4	24 300	0.43	0.48	0.03	12.5	68.1
Mo-02	25.0	20.0	68.0	29 300	40.7	45.5	135	34 600	0.90	0.86	0.05	13.3	61.3
Mo-03	21.0	13.3	60.0	28 000	35.0	30.3	119	32 100	0.46	0.61	0.04	13.5	65.3
Be-03	35.7	23.3	65.7	27 700	107	65.0	342	35 800	0.74	0.89	0.07	12.9	63.7
Be-16	54.7	33.3	105	37 300	145	85.7	433	43 300	1.54	1.30	0.12	14.3	57.5
Br-01	38.3	26.7	80.7	27 700	106	97.5	242	33 700	0.56	0.76	0.05	13.7	63.1
Br-06	45.3	26.7	81.3	28 000	142	100	318	33 500	0.75	0.91	0.06	13.5	62.0
Br-11	59.3	36.7	112	32 700	180	151	423	39 000	0.84	1.26	0.06	14.0	61.6

conducted. This generally resulted in greater coefficients of determination (r^2). Since our goal was to obtain the best overall single predictor among the fractions and normalized parameters, all subsequent statistical analyses were bivariate. The correlation coefficient values (r) were calculated in each case and the data were examined graphically to verify spread and linearity. For all the calculations, mean values for the tissues (or organism) at each station were used. Rather than present all the statistical parameters, we shall limit the following discussion to the general trends observed for each metal (Tables 4–6).

For Cu (Table 4), significant correlations generally occurred between the metal concentration in the soft tissues of *E. complanata* and [Cu(F2)] or [Cu(F3)] ; correlation coefficients were not calculated with [Cu(F1)], as the latter values were lower than or close to the analytical detection limit (Table 1). The r values increased when the various Cu concentrations in the sediments were normalized with respect to the organic content in the sediments or, more importantly, with respect to [Fe(F3)]; when normalization of the trace metal concentrations in the sediments was effected, higher correlation coefficients were obtained if [Cu(S3)] was considered in the calculation instead of [Cu(F2)] or [Cu(F3)], where [Cu(S3)] is the sum of [Cu(F1)], [Cu(F2)], and [Cu(F3)]. The ratio [Cu(S3)]/[Fe(F3)] in the sediments (Fig. 3) was the best predictor of the Cu concentration in the whole mollusc.

A slightly different trend was observed for Zn (Table 5). Few significant correlations were observed between Zn concentration in the soft tissues of the bivalves and [Zn(F1)], [Zn(F2)], or [Zn(F3)]; the correlations with [Zn(F4)] were the most numerous and statistically significant (Table 5). In contrast with Cu, normalizing the various Zn concentrations with respect to the organic carbon content of the sediments systematically decreased the r value; however, normalizing [Zn(F4)] or [Zn(S3)] with respect to [Fe(F3)] did increase the correlation coefficients. The ratio [Zn(F4)]/[Zn(F3)] in the sediments (Fig. 4) was the best predictor of the Zn concentration in the whole mollusc.

For Pb, a statistically significant correlation coefficient was found between the metal level in the gills and [Pb(F3)] (Table 6); correlation coefficients involving either [Pb(F1)] or the foot and adductor muscles were not calculated, as the Pb concentrations were lower than the analytical detection limits. The r values increased when the various Pb concentrations in the sediments were normalized with respect to the organic content in the sediment or, more importantly, with respect to [Fe(F3)]. When normalization was effected, higher correlation coefficients were obtained when [Pb(S3)] was considered in the calculation instead of [Pb(F3)].

Discussion

Copper, Pb, and Zn levels in various tissues of *E. complanata* (or in the whole organism) can be predicted, in the study area, from trace metal concentrations extracted from the sediments. As expected, correlations are better for relatively easily extractible metals (e.g. F2, F3, F4) than for more tightly bound metals (residual; F5), or for the total trace metal concentrations (S5). This suggests that the availability of a particular metal is inversely related to its binding strength to the various substrates in the sediments. Extracting the sediments and normalizing with respect to sediment components (e.g. [Fe(F3)], organic carbon) increased the explained variance in accumulated metals in the tissues of the organisms over that explained by the total metal concentrations in the sediments. For example, using [M(S3)]/[Fe(F3)] instead of [M(S5)] as the predictor increased r^2 by 33%

TABLE 2. Metal concentrations in the tissues of *E. complanata* from various sampling sites. Fo, foot; Mu, adductor muscle; VM, visceral mass; He, hepatopancreas; Gi, gills; Ma, mantle; RO, reconstituted organism (calculated with equation 1); ND, not detectable.

Site	Organ	Mean ± SD (μg/g dry weight)				
		Cu	Pb	Zn	Fe	Mn
Mo-01	Fo	5.4±4.8	ND	127±36	85±21	144
	Mu	2.4±0.6	ND	83±13	67±27	60±5
	VM	8.5±2.0	5.8±3.6	78±11	390±270	320±280
	He	29.0±13.2	13.0	123±23	400±390	89±77
	Gi	29.5±19.4	18.8±6.9	265±45	840±360	5000±300
	Ma	17.6±9.5	13.9±4.2	173±19	3 700±1300	3000±3400
	RO	13.8±5.0	—	132±15	925±460	1330±450
Mo-02	Fo	5.4±1.8	ND	140±8	109±71	103
	Mu	2.7±1.1	ND	76±9	82±23	36
	VM	10.5±2.9	ND	84±9	770±290	147
	He	28.8±7.8	14.7±3.5	121±7	670±90	103
	Gi	27.8±11.9	12.9±1.9	280±9	1 450±740	4950
	Ma	11.8±0.8	14.4±4.1	160±14	5 600±1200	1560
	RO	13.6±2.3	—	136±4	1 470±500	1270
Mo-03	Fo	7.7±3.2	ND	130±31	119±32	52±15
	Mu	4.6±1.0	ND	95±10	300±130	113±32
	VM	11.8±3.6	4.8±1.8	98±10	1 080±500	650±60
	He	32.0±3.2	31	170±24	1 130±190	178±11
	Gi	28.2±16.4	12.2±2.8	320±90	2 590±1000	8600±3380
	Ma	18.4±2.0	21.1±9.7	280±56	10 400±400	5510±1240
	RO	16.0±2.5	—	180±50	3 000±330	2800±1250
Be-03	Fo	6.9±1.3	ND	144±10	114±66	47±17
	Mu	3.4±0.9	ND	88±10	115±71	74±31
	VM	11.1±3.3	1.4	90±12	650±150	400±140
	He	32.0±5.3	ND	119±10	880±340	230±95
	Gi	58.8±11.2	6.3	790±270	1 610±310	8800±1380
	Ma	43.9±6.9	7.7±1.7	275±87	4 070±1440	2580±610
	RO	23.3±3.4	—	202±38	1 220±260	1570±170
Be-16	Fo	7.9±1.9	ND	148±28	390±310	166±141
	Mu	7.7±5.8	ND	119±22	280±142	184±116
	VM	11.7±3.4	6.3	160±65	1 120±780	540±270
	He	37.5±8.2	13.1	208±23	1 490±810	260±84
	Gi	30.3±6.0	14.4±1.3	1360±890	3 440±720	8720±1940
	Ma	69.8±44.8	21.1±5.2	1190±980	11 100±2100	8590±2120
	RO	28.2±9.7	—	535±320	3 340±460	3230±510
Br-01	Fo	12.7±7.9	5.5±1.4	141±15	108±27	43±11
	Mu	11.7±7.7	3.8	100±11	135±56	59±19
	VM	16.5±4.4	6.9±0.8	114±6	585±212	286±136
	He	44.5±1.3	14.3±7.2	172±8	755±200	153±63
	Gi	214±110	36.0±2.7	1060±370	2 560±640	8660±1000
	Ma	94±90	33.3±8.0	332±117	3 420±1000	2260±350
	RO	59.1±22.3	—	295±43	1 160±180	1830±170
Br-06	Fo	13.1±6.9	ND	154±18	203±174	105±130
	Mu	10.7±3.9	ND	103±12	262±173	134±101
	VM	16.1±5.9	6.0±2.0	129±34	570±245	240±200
	He	72.9±39.2	15.3	187±9	800±560	140±80
	Gi	132±73	35.4±9.8	1030±140	2 480±1250	7120±640
	Ma	81.7±33.0	35.6±10.7	425±103	5 370±1240	2900±1400
	RO	46.8±8.3	—	320±46	1 520±180	1660±210
Br-11	Fo	12.8±2.9	ND	141±18	164±59	61±30
	Mu	5.6±2.1	ND	98±9	136±128	67±45
	VM	23.5	5.5	103	438	264
	He	56.6	20.2	187	880	199
	Gi	203	39.8	832	2 700	9870
	Ma	134	25.2	382	3 570	4290
	RO	68.2	—	272	1 260	2380

TABLE 3. Relative contributions (%) of various tissues to the total metal burden in *E. complanata*.

Tissue	Cu	Zn	Fe	Mn	Relative dry weight
Gills	36±13	40±11	21±9	61±14	15±4
Mantle	28±13	25±11	58±11	31±14	17±3
Hepatopancreas	12±5	5±2	4±1	0.7±0.3	8±2
Visceral mass	18±7	16±4	15±4	7±3	36±5
Adductor muscle	3±1	7±2	1.4±0.4	0.6±0.1	15±2
Foot	3±1	7±2	0.8±0.2	0.3±0.2	10±2

for both Pb in the gills and Cu in the reconstituted organisms; using [Zn(F4)]/[Fe(F3)] increased r^2 by 21% over [Zn(S5)]. Based on the statistical analysis, however, it is difficult to associate bioavailability of sediment-bound trace metals to well-identified phases of sediment. Due to the restricted geographical area examined in this study, intercorrelations between the various metal fractions in the sediments are frequently observed, and for a given trace metal, tissue levels are often correlated with several metal fractions in the sediments. The difficulty in identifying key geochemical phases is compounded by the fact that the chemical extractions are not completely selective. To fully evaluate the predictive power of the various metal fractions in the sediments, studies should be conducted in watersheds of diverse geological characteristics.

The consistent increase in the correlation coefficient values that is observed when the trace metal concentrations extracted from the sediments are normalized with respect to [Fe(F3)] suggests a "protective" or "competitive" role for Fe in the processes leading to the accumulation of trace metals by *E. complanata*. This Fe, extracted with hydroxylamine hydrochloride, presumably originates mainly from amorphous iron oxyhydroxides; well-crystallized iron oxides (e.g. goethite, hematite) are not easily attacked under these conditions (Rapin and Forstner 1983). The accumulation of Pb (Luoma and Bryan 1978) and As (Langston 1980) in *Scrobicularia plana* in various English estuaries was also well predicted by the ratios [Pb]/[Fe] and [As]/[Fe], respectively, in hydrochloric acid extracts of surface sediments; the Fe extracted with 1 mol HCl/L was considered by these researchers to be mainly amorphous iron oxides.

Depending on the main route for accumulation of trace metals in *E. complanata*, at least three mechanisms could explain an inverse dependence of trace metal accumulation upon the iron oxyhydroxide concentration in the sediment.

Mechanism 1

If it is assumed that the major route for the accumulation of

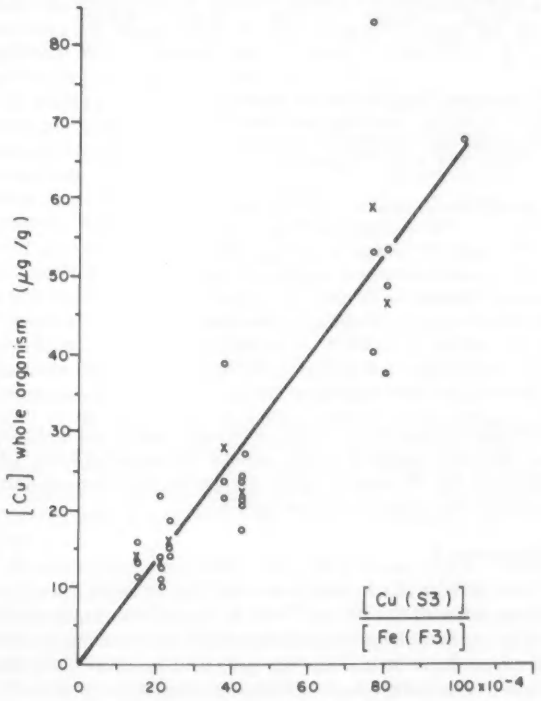

FIG. 3. Relationship between the concentration of Cu in *E. complanata* and the ratio [Cu(S3)]/[Fe(F3)] in the sediments ($Y = 6854X - 0.02$; $r^2 = 0.95$). ○, individual specimens; ×, mean value at each station.

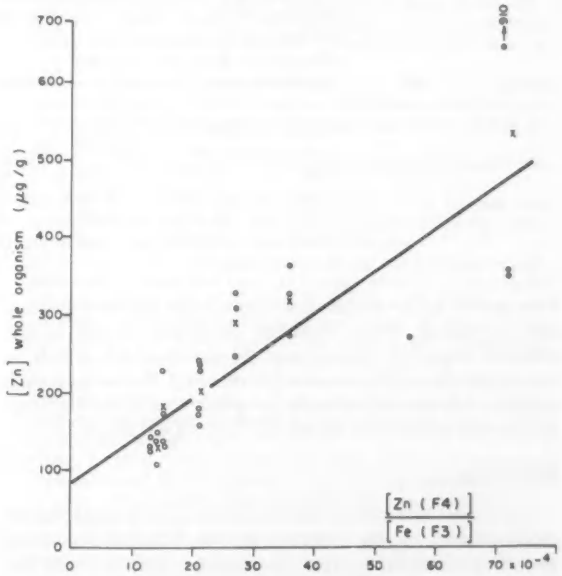

FIG. 4. Relationship between the concentration of Zn in *E. complanata* and the ratio [Zn(F4)]/[Fe(F3)] in the sediments ($Y = 54256X + 86$; $r^2 = 0.79$). ○, individual specimens; ×, mean value at each station.

TABLE 4. Correlation coefficients between Cu in sediments and Cu in various tissues of *E. complanata*. Values significantly different from zero at the 5% (regular type) and 1% levels (italicized) are distinguished from those not significantly different from zero at the 5% level (in parentheses). (S3) = (F1) + (F2) + (F3). Fo, foot; Mu, adductor muscle; VM, visceral mass; He, hepatopancreas; Gi, gills; Ma, mantle; RO, reconstituted organism (calculated with equation 1).

Tissue	Cu(F2)	Cu(F3)	Cu(S3)	Cu(S5)	Cu(S3)/ Fe(F3)	Cu(S3)/ organic C
Fo	*0.846*	*0.852*	*0.872*	0.734	*0.945*	*0.898*
Mu	(0.595)	(0.463)	(0.548)	(0.556)	(0.652)	(0.617)
VM	0.826	*0.961*	*0.913*	0.757	*0.930*	*0.853*
He	0.805	(0.704)	0.778	0.718	*0.841*	0.732
Gi	0.790	*0.915*	*0.873*	(0.633)	*0.931*	*0.954*
Ma	*0.921*	*0.927*	*0.947*	*0.905*	*0.956*	*0.850*
RO	*0.873*	*0.943*	*0.930*	0.788	*0.974*	*0.921*

TABLE 5. Correlation coefficients between Zn in sediments and Zn in various tissues of *E. complanata*. Values significantly different from zero at the 5% (regular type) and 1% (italicized) levels are distinguished from those not significantly different from zero at the 5% level (in parentheses). (S3) = (F1) + (F2) + (F3). Fo, foot; Mu, adductor muscle; VM, visceral mass; He, hepatopancreas; Gi, gills; Ma, mantle; RO, reconstituted organism (calculated with equation 1).

Tissue	Zn(F1)	Zn(F2)	Zn(F3)	Zn(F4)	Zn(S3)	Zn(S5)	Zn(F4)/ organic C	Zn(S3)/ organic C	Zn(F4)/ Fe(F3)	Zn(S3)/ Fe(F3)
Fo	0.753	(0.690)	0.746	(0.599)	0.745	0.742	(0.541)	(0.603)	(0.556)	0.759
Mu	(0.548)	(0.586)	(0.664)	0.784	(0.648)	(0.692)	(0.636)	(0.367)	*0.829*	0.770
VM	(0.541)	(0.579)	(0.639)	0.771	(0.628)	(0.677)	(0.528)	(0.277)	0.814	0.748
He	(0.355)	(0.396)	(0.578)	0.737	(0.528)	(0.610)	(0.689)	(0.295)	0.803	0.688
Gi	0.793	0.753	*0.845*	0.802	*0.833*	*0.847*	0.727	(0.640)	0.793	*0.894*
Ma	(0.523)	(0.638)	(0.606)	0.829	(0.618)	(0.665)	(0.434)	(0.127)	*0.860*	(0.698)
RO	(0.621)	(0.671)	0.722	*0.860*	0.712	0.760	(0.600)	(0.331)	*0.889*	0.814

TABLE 6. Correlation coefficients between Pb in sediments and Pb in various tissues of *E. complanata*. Values significantly different from zero at the 5% (regular type) and 1% (italicized) levels are distinguished from those not significantly different from zero at the 5% level (in parentheses). (S3) = (F1) + (F2) + (F3). VM, visceral mass; He, hepatopancreas; Gi, gills; Ma, mantle.

Tissue	Pb(F2)	Pb(F3)	Pb(S3)	Pb(S5)	Pb(S3)/ Fe(F3)	Pb(S3)/ organic C
VM	(0.334)	(0.291)	(0.300)	(0.264)	(0.524)	(0.341)
He	(−0.304)	(−0.086)	(−0.136)	(−0.160)	(−0.187)	(−0.053)
Gi	(0.648)	*0.873*	*0.856*	0.774	*0.964*	*0.916*
Ma	(0.545)	(0.631)	(0.638)	(0.573)	0.751	0.706

trace metals in *E. complanata* involves the digestive system, and that acidic and/or reducing conditions prevail in the intestinal tract, one can envisage the simultaneous solubilization of both Fe and trace metals (Cu, Pb, Zn). The Fe could then compete with the trace metals for uptake sites in the digestive system and reduce their uptake (cf. Luoma and Bryan 1978).

Mechanism 2

If it is again assumed that accumulation of trace metals occurs predominantly via the digestive system, but that conditions prevailing within the gut (pH, pε, residence time) are such that the iron oxyhydroxides are not solubilized but remain in particulate form, then the unreacted iron oxyhydroxides could compete with the uptake sites in the intestinal tract for the solubilized trace metals. Higher levels of iron oxyhydroxides in the ingested sediment would reduce the concentrations of dissolved Cu, Pb, and Zn in the gut during digestion and thus result in lower uptake of these metals.

Mechanism 3

Alternatively, and perhaps more likely, the main route for accumulation of Cu, Pb, and Zn by *E. complanata* may involve not the ingestion of particulate material but rather the uptake of dissolved trace metals via the gills and mantle. Then the protective role of the iron oxyhydroxides could be explained by invoking adsorption in the *external* medium as the principal factor controlling the dissolved trace metal concentrations to which the organism is exposed. This adsorption can be

characterized in a simplified manner by

$$(2)\quad M + S(k) = MS(k)$$

$$(3)\quad D(k) = \frac{[MS(k)]}{[M][S(k)]}$$

where D(k) is a distribution coefficient and [MS(k)], [M], and [S(k)] represent, respectively, the concentration of adsorbed trace metal M, the concentration of dissolved trace metal, and the number of sites available for adsorption on the substrate *k* (cf. Oakley et al. 1981). Clearly, as the concentration of adsorbing substrate ([S(k)] ≡ [Fe(F3)]) increases, the concentration of dissolved trace metal will decrease.

The high levels of trace metals associated with the gills and mantle (Table 2) and the high contribution of these two organs to the body burden (Table 3) suggest that uptake (or extracellular binding) of dissolved trace metals is an important route of accumulation of these elements in *E. complanata*. These two organs are physiologically remote from the digestive system of the organism, are in contact with large amounts of water necessary for feeding and respiration (approximately tens of litres per day; Mellanby 1938; Mohlenberg and Riisgard 1979), and have large surface areas. The above reasoning, coupled with the fact that most filter feeders selectively feed on phytoplankton and minimize their digestion of inorganic sediments, and with the observed inverse relationship between trace metal levels in the gills or mantle and [Fe(F3)], lends support to mechanism 3. The possibility of a direct uptake of particulate trace metals by the gills and mantle by endocytosis cannot be rejected (Brown 1982); Coombs (1980) has described such a process, i.e. engulfment of the particulate metal by the epithelial cell membrane, which then pinches off to form membrane-limited vesicles within the cell.

In addition to the iron oxyhydroxides discussed above, other sediment constituents might be expected to influence the availability of trace metals to benthic organisms; for example, manganese oxides and organic matter often constitute important sinks for trace metals in the oxidized layer of freshwater sediments (Tessier et al. 1980, 1982; Forstner and Wittman 1981; Lion et al. 1982). Normalizing the Cu and Pb levels in the sediments with respect to the organic carbon content of the sediments does indeed increase the correlation coefficients (Tables 4–6). Similar results were reported by Langston (1982) who found that Hg accumulation in *Scrobicularia plana* and *Macoma balthica* was best predicted by the ratio of the Hg concentration extracted from the sediment with concentrated HNO_3 to the organic content (ashing at 400°C) of the sediments. It should be noted that the apparent controlling effect of organic matter is observed for trace metals for which the stability constants with organic ligands are high (Hg, Cu, Pb) and not for other trace metals with lower stability constants (e.g. Zn; Table 5).

In conclusion, the present study demonstrated that (1) trace metal accumulation in *E. complanata* in the Rouyn–Noranda area is best related to one or more fractions of the total metal load (rather than to the latter) and (2) metal accumulation is also influenced by the protective or competitive effect of sediment constituents, notably amorphous iron oxyhydroxides. These observations confirm those made previously for benthic organisms in the estuarine environment (Luoma and Bryan 1978; Langston 1980).

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Particulate Allochthonous Inputs: Relationships with Stream Size in an Undisturbed Watershed[1]

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Conners, M. E., and R. J. Naiman. 1984. Particulate allochthonous inputs: relationships with stream size in an undisturbed watershed. Can. J. Fish. Aquat. Sci. 41: 1473–1484.

Four streams of a pristine boreal watershed in eastern Quebec were examined to assess the composition, seasonal pattern, and ash-free dry mass (AFDM) of particulate allochthonous input. Study sites on streams ranging from first to sixth order exhibited significant differences in the types and magnitude of litter input. Composition of litterfall varied according to vegetation and physical structure of the riparian zone. Although the forest in the watershed is dominated by conifers, deciduous leaves dominated annual budgets and seasonal input patterns. Annual litterfall per unit area of stream surface declined exponentially with increasing stream order (r^2 = 0.98) from 307–534 g AFDM·m^{-2}·yr^{-1} in a first-order stream to 15–17 g AFDM·m^{-2}·yr^{-1} in a sixth-order stream. Lateral inputs of 25–70 g AFDM·m^{-1}·yr^{-1} were not related to stream size, but were strongly influenced by riparian structure and entrainment of organic matter during spring flooding. Total input per unit stream length increased logarithmically with increasing order (r^2 = 0.87), approaching 1000 g AFDM·m^{-1}·yr^{-1} in the fifth- and sixth-order streams. A watershed budget calculated from these results estimates a total input of 729 t·yr^{-1} to streams of this 673-km^2 watershed. When particulate allochthonous input is compared with primary production measurements in these streams, 81–95% of the organic carbon supplied to the first- and second-order streams is allochthonous, but 85–95% of the supply to the fifth- and sixth-order streams is autochthonous. While these trends agree with predictions of the synthetic continuum model, the importance of local effects in our results illustrates the need for a more mechanistic approch in allochthonous input research.

Quatre cours d'eau d'un bassin versant boréal non perturbé de l'est du Québec ont fait l'objet d'une étude visant à déterminer la composition, l'allure saisonnière et la masse sèche sans cendre (MSSC) des apports allochtones particulaires. Les endroits étudiés de cours d'eau, dont l'ordre allait d'un à six, présentaient des différences appréciables quant au type et à l'importance de l'apport de litière. La composition de l'apport de litière était fonction de la végétation et de la structure physique des rives. La forêt du bassin versant est dominée par les conifères, mais les feuilles de décidus s'avéraient dominantes dans les bilans annuels et les allures saisonnières des apports. L'apport annuel par précipitation de litière par unité de surface de cours d'eau diminuait de façon exponentielle en fonction de l'accroissement de l'ordre du cours d'eau (r^2 = 0,98) ; il était de 307–534 g MSSC·m^{-2}·an^{-1} dans un cours d'eau d'ordre un et de 15–17 g MSSC·m^{-2}·an^{-1} dans un cours d'eau d'ordre six. Les apports latéraux de 25–70 g MSSC·m^{-1}·an^{-1} n'étaient pas liés à la taille du cours d'eau, mais dépendaient fortement de la structure des rives et de l'entraînement de la matière organique pendant la crue de printemps. L'apport total par unité de longueur du cours d'eau augmentait de façon logarithmique en fonction de l'accroissement de l'ordre du cours d'eau (r^2 = 0,87) pour s'approcher d'une valeur de 1000 g MSSC·m^{-1}·an^{-1} dans les cours d'eau d'ordre cinq ou six. Le bilan du bassin versant, estimé à partir de ces résultats, indique un apport de 729 t·an^{-1} pour les cours d'eau de ce bassin de 673 km^2. La comparaison des apports allochtones particulaires avec la production primaire mesurée dans les cours d'eau indique que 81–95 % du carbone organique pénétrant dans les cours d'eau d'ordre un et deux est d'origine allochtone tandis que 85 à 95 % de celui des cours d'eau d'ordre cinq et six est d'origine autochtone. Ces tendances correspondent aux prévisions du modèle de continuité synthétique, mais l'importance des effets locaux indiqués par nos résultats illustre bien la nécessité de faire appel à une approche plus empirique pour la recherche sur les apports allochtones.

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Fragments of riparian vegetation entering rivers and streams form an important link between terrestrial and aquatic productivity. This particulate litter input, along with groundwater inputs of dissolved organic matter, determines the allochthonous supply of reduced organic carbon available to stream food webs. The balance between allochthonous input and autochthonous primary production plays a key role in structuring the invertebrate community and metabolic dynamics of the stream ecosystem (Cummins 1974). Thus local, specific information on the types and amount of particulate allochthonous input is essential for energetic or nutrient budgets of individual streams, while an overall understanding of factors and processes controlling this input is one important goal of stream ecosystem research.

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Most available data on allochthonous inputs come from single-site studies of small streams in forested watersheds (see Anderson and Sedell 1979 for a partial review). A recent trend in stream research, however, is to examine stream processes in a watershed perspective, emphasizing changes in dynamics over the gradient from headwater streams to large rivers (Vannote et al. 1980; Naiman and Sedell 1981; Minshall et al. 1983; Naiman 1983a). In drawing a generalized picture of expected changes along this gradient, these studies make the assumption that the importance of large particulate allochthonous input declines with increasing stream size. Although some evidence in support of this assumption can be found in the literature (Cummins 1974; Fisher 1977), quantitative data confirming this trend within a single drainage network have not been presented.

The relative importance of laterally transported litter to total particulate income also remains incompletely understood. Reported values for the percentage of total particulate input received through lateral transport range from negligible (Fisher 1977) to 66% (Triska et al. 1982). Differences between biomes in landform and soils obviously determine much of this variability, but no investigations have been made as to stream size associated changes within a biome.

Finally, emphasis on small streams has also resulted in a lack of information on the spatial patterns of particulate input to large streams. It is widely assumed that midchannel surfaces collect less litter than those directly beneath the bordering canopy, but quantitative investigations of this difference have been rare (Matthews and Kowalczewski 1969).

This study examines the composition, timing, and magnitude of particulate allochthonous inputs to four streams of a pristine boreal watershed in eastern Quebec. The streams range from first to sixth order with completely closed to fully open forest canopies. The objectives of this investigation were to (1) characterize allochthonous input to each study site, including the contribution of lateral inputs and the spatial pattern of litterfall to wide stream channels, (2) examine annual litter inputs along the continuum of stream size, and (3) estimate total particulate loading to the drainage network. This study is part of a multifaceted project investigating stream processes and watershed dynamics in Quebec (Naiman 1982a, 1983a, 1983b, 1983c; Melillo et al. 1983; Naiman and Sedell 1981).

Study Sites

The Matamek River watershed is located approximately 25 km east of Sept-Îles, Quebec, on the North Shore of the Gulf of St. Lawrence. The terrain is pristine boreal forest with no history of logging or other disturbance. Since 1966 the entire watershed has been protected by the Province of Quebec as an environmental area where only scientific research and controlled fur trapping are permitted.

Streams in this region are typically shallow and low gradient, low in nutrients but high in humic acids and dissolved organic carbon. A heavy pack of ice and snow develops by December and covers the streams until the end of March, producing a strong spring freshet in late April or May (Naiman 1982a). Forest vegetation in the watershed is black and white spruce (*Picea mariana* and *P. glauca*) and balsam fir (*Abies balsamea*), with some paper birch (*Betula papyrifera*) and trembling aspen (*Populus tremuloides*). Speckled alder (*Alnus rugosa*) is common in riparian zones.

Two terrestrial plots and five stream sites were included in the study (Fig. 1), representing typical forest areas and a range of stream sizes found in the Matamek River drainage network. Detailed descriptions of these streams and of the Matamek region in general may be found in Naiman (1982a, 1983a).

First Choice Creek drains a small coastal watershed adjacent to the Matamek River basin (Fig. 1). There is substantial riparian development along this first-order stream; the channel is narrow and the forest canopy closes completely over the streambed.

Beaver Creek is a second-order stream whose upstream reaches have been dammed into a series of abandoned beaver (*Castor canadensis*) ponds. One study site was situated along a narrow riffle-pool segment just before the stream enters the Matamek River (Fig. 1). Dense riparian stands of alder and paper birch form an almost fully closed canopy over this segment, although some direct sunlight reaches the water at midday. This site was considered representative of second-order streams for watershed analyses. A second study site was established at a beaver pond upstream, where feeding and dam building activities have created an open meadow covered primarily by grasses on either side of the pond (shown by the broken line in Fig. 1). The combined effects of increased stream width and cleared riparian vegetation at this site provide a predominantly open forest canopy.

The study site on the fifth-order Muskrat River was in a low-gradient reach just before its confluence with the Matamek River (Fig. 1). The channel is deep, straight, and relatively narrow, with square-cut banks bordered by a dense spruce–fir forest and a narrow band of alder. Almost all of the water surface is open to sunlight.

The Matamek River is a shallow, rapidly flowing sixth-order stream with a stair-step morphology of waterfalls from Matamek Lake to the Gulf of St. Lawrence. The study site was located at the confluence of Beaver Creek just below Third Falls (Fig. 1), where the river is broad and has a completely open canopy. The shoreline is bordered by alder and mixed birch–spruce forest.

The two terrestrial study sites were located 10–15 m west of First Choice Creek and in the forest beyond the riparian zone near the downstream end of Beaver Creek (Fig. 1). A 1200-m^2 plot at each site was surveyed to determine forest composition and density (J. M. Melillo and R. J. Naiman, unpubl. data). The plot near First Choice Creek had a density of 1315 stems·ha^{-1} and a total basal area of 34.22 $m^2 \cdot ha^{-1}$, of which 37.3% was balsam fir, 32.1% paper birch, 25.7% black spruce, and 4.9% trembling aspen. The plot near Beaver Creek had a density of 2046 stems·ha^{-1} and a 47.95 $m^2 \cdot ha^{-1}$ total basal area composed of 52.8% spruce, 40.2% birch, and 5.3% fir.

Materials and Methods

Litterfall at the terrestrial sites and along the banks of First Choice Creek and Beaver Creek was collected in 0.089-m^2 plastic tubs. Ten replicate samplers at each site were placed to cover the full range of vegetational types (Fig. 1). Bray and Gorham (1964), Gosz et al. (1972), and Merriam et al. (1982) emphasized that small area samplers are inadequate for measuring inputs of large woody debris. Wood input to these litter traps was assumed to describe only twig and small branch fall.

On the Muskrat River and Matamek River, innertubes were moored to concrete blocks in a pattern of bank-to-bank transects with numbered trap positions at fixed distances from shore (Fig. 1). Sampling tubs were then tied into the innertubes, giving floating "array traps." Five transects were established on each

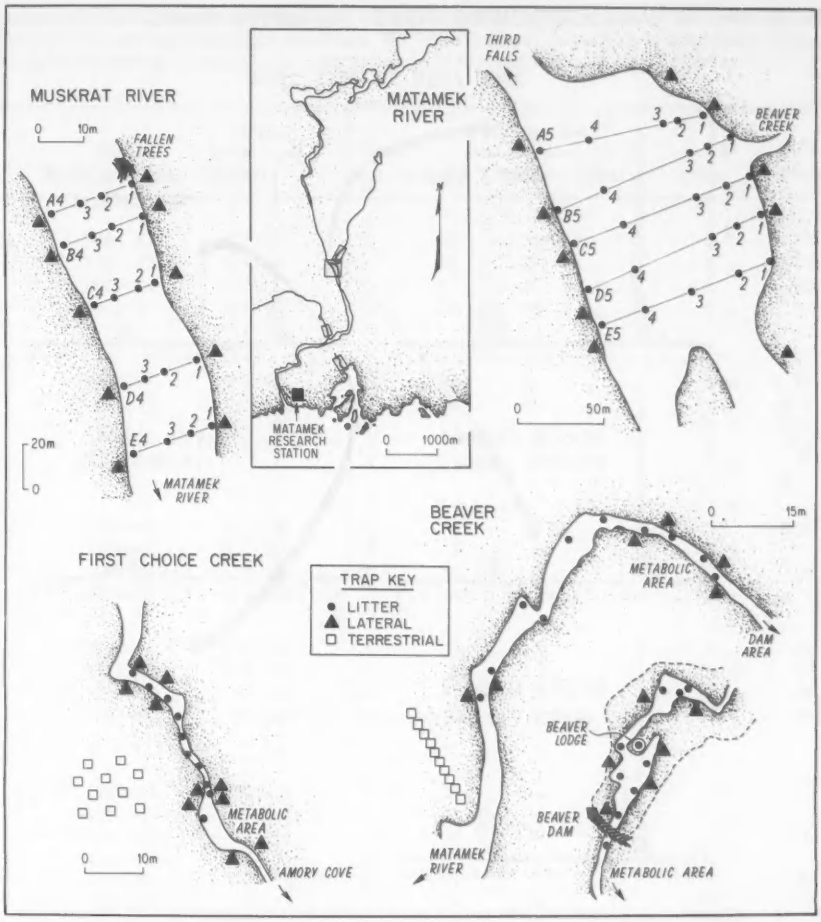

FIG. 1. Location of study sites and trap placement. The Matamek Research Station is at about 50°10′N, 65°00′W.

river, with the trap positions on each transect corresponding as closely as possible. The replicate transects were lettered A–E, and trap positions across the transect were numbered (1–4 on the Muskrat River, 1–5 on the Matamek River). For calculations, the five transects were treated as replicates and mean input to each numbered trap position was determined.

Lateral input was measured at all five stream sites with samplers constructed from wood and 0.5-cm-mesh galvanized screening. Triangular endpieces joined by back and bottom sides of screening formed open-sided prisms 40 cm long and 26 cm deep. A lip of screening along the bottom of the open side retained material in the traps. Six to 10 traps were placed close to the stream bank at each site with open sides facing away from the water; the traps were placed in locations where accumulated litter would normally be entrained by the stream.

Litter traps were set in May 1980 and emptied and reset periodically through May 1982. Traps were emptied weekly, biweekly, or monthly depending on intensity of litterfall. Wintertime allochthonous inputs were measured at spring thaw by collecting traps set the preceding November. Traps were occasionally missing or overturned, but only once were all traps from a collection lost.

On each sampling date, material in the traps was collected on a 1-mm-mesh sieve, put into plastic bags, and frozen. In the laboratory, thawed samples were sorted into leaf, wood, bark, and needle fractions and identified to species whenever possible. All fractions were dried at 80°C and weighed to the nearest 0.01 g. During the first 3 mo of the study each litter fraction was ashed for 4 h at 525°C. The mean ash content of the fractions ranged from 0.8 to 9.1% of dry weight. Since the variability in ash content was small, these means were used to convert dry mass (DM) to ash-free dry mass (AFDM) for the entire data set. No attempt was made to correct these weights for leaching loss of soluble organics prior to collection. For each sampling interval, the mean input of each of seven major litter types and the mean total input were determined from the 5–10 replicate traps.

Annual particulate input to each study site was estimated for May 1, 1980–April 30, 1981, and May 1, 1981–April 30, 1982. Sampling interval lengths were adjusted to cover exactly 1 yr and the annual income computed as the weighted sum of interval means. Mean input values from adjacent intervals or the equivalent intervals in the other study years were used for any period where direct data were absent.

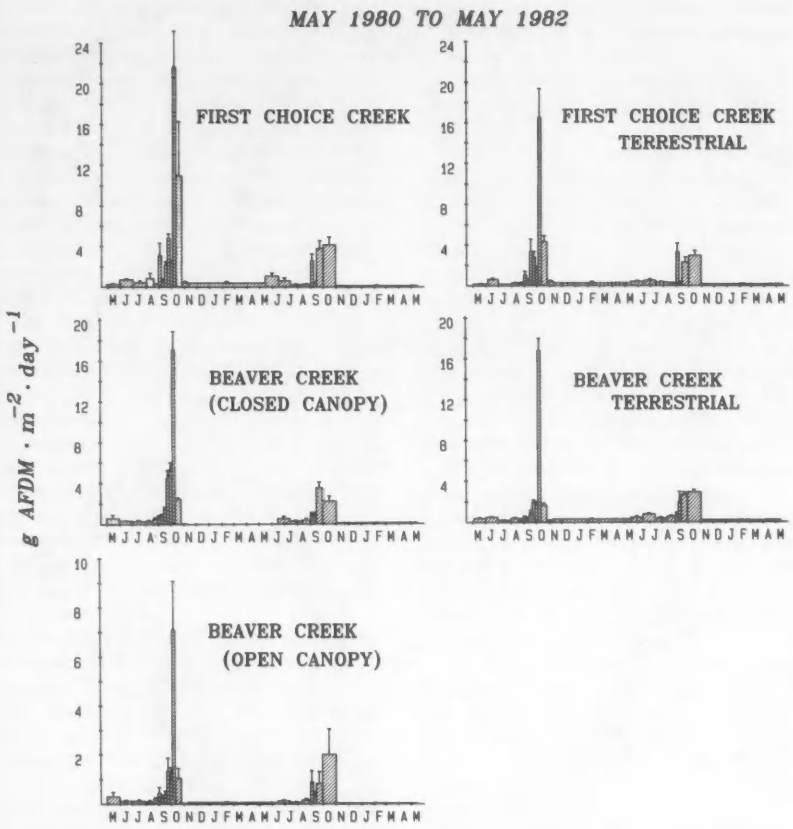
ALLOCHTHONOUS INPUT - LITTER TRAPS
MAY 1980 TO MAY 1982
FIRST CHOICE CREEK
FIRST CHOICE CREEK TERRESTRIAL
BEAVER CREEK (CLOSED CANOPY)
BEAVER CREEK TERRESTRIAL
BEAVER CREEK (OPEN CANOPY)
g AFDM · m⁻² · day⁻¹
M J J A S O N D J F M A M J J A S O N D J F M A M

FIG. 2. Allochthonous input to the litter traps at the terrestrial and small stream sites. Each histogram cell represents one sampling interval: width indicates span of the interval, height indicates mean input to 10 replicate traps. Bars show standard errors of the mean.

Total litterfall to a 1-m-long stream segment was also calculated for each of the study sites by multiplying the per square metre input rate by stream width. Total lateral input at each site was multiplied by the length of shoreline associated with the segment. These two estimates, when combined, represent total loading to the segment. Shoreline length to stream length ratios of our study streams were measured from previously published maps (for First Choice Creek and Beaver Creek: Naiman 1982b; for the Matamek River: Frenette and Julien 1981). No detailed map was available for the Muskrat River; it was assigned a shore to stream length ratio of 2.10 on the basis of personal observations.

For watershed calculations, total input per unit stream length was estimated for stream orders 1–6 by the same method used for specific study sites. Direct litter inputs to each stream order were estimated from the regression of the combined 2-yr data on stream order. Lateral input was assumed to be constant throughout the watershed at 45 g AFDM·m^{-1}·yr^{-1} (the average from all study sites). Fourth-order streams were arbitrarily assigned a shoreline length to stream length ratio of 2.28, and fifth-order streams were assigned a ratio of 2.30, since the Muskrat River is atypically straight compared with other streams in the watershed. Mean widths typical of each stream order were calculated from the following relationship (Naiman 1983a):

$$\log_{10}(\text{mean width in metres}) = 2.93 \log_{10}(\text{stream order}) - 0.57.$$

Total input estimates calculated from these approximations were then multiplied by the total length of each stream order in the watershed.

Results and Discussion

Input Mass and Composition

Direct input — headwater streams

The magnitude and seasonal variation of litterfall at the terrestrial and headwater stream sites (First Choice Creek and Beaver Creek) were similar to those reported from other boreal forests (Merriam et al. 1982; Iversen et al. 1982). Mean input values for the 10 traps at each site ranged from summer minima of 0.06–0.37 g AFDM·m^{-2}·d^{-1} to autumn peaks of 7.1–21.6 g AFDM·m^{-2}·d^{-1} (Fig. 2). As is commonly reported in

TABLE 1. Annual estimates of particulate allochthonous input and its composition. Inputs are divided into hardwood leaves, twigs and woody debris, conifer needles and cones, and hardwood seeds and catkins. The "other" category includes unidentified leaves, bark, and herbaceous litter. Standard errors of the input estimates are in parentheses. Units for terrestrial sites and litter traps are g AFDM·m^{-2}·yr^{-1} and for lateral traps are g AFDM·m^{-1}·yr^{-1}.

Site	Study year	Mean annual input (±SE)	% of annual input					% of leaf input		
			Leaves	Wood	Needles	Catkins	Other	Birch	Alder	Aspen
Terrestrial sites										
First Choice Creek	1980–81	327 (33)	58	15	26	2	1	85	4	11
	1981–82	229 (19)	51	13	23	6	7	75	3	22
Beaver Creek	1980–81	280 (15)	60	11	26	1	2	92	5	3
	1981–82	255 (6)	46	13	28	5	8	91	2	6
Stream sites										
First Choice Creek										
Litter traps	1980–81	534 (83)	46	33	18	2	1	79	2	17
	1981–82	307 (31)	52	19	13	5	11	71	1	29
Lateral traps	1980–81	53 (10)	67	13	4	1	14	83	1	16
	1981–82	51 (4)	77	13	5	1	5	78	1	21
Beaver Creek										
Closed Canopy										
Litter traps	1980–81	312 (19)	84	8	5	1	1	36	55	9
	1981–82	212 (26)	67	9	4	4	15	49	39	11
Lateral traps	1980–81	53 (16)	56	30	1	1	10	68	22	8
	1981–82	55 (10)	83	8	2	1	5	72	16	11
Open Canopy										
Litter traps	1980–81	120 (17)	79	9	5	1	6	47	47	3
	1981–82	103 (36)	73	5	2	3	16	42	52	5
Lateral traps	1980–81	30 (16)	24	32	3	1	41	47	48	5
	1981–82	26 (6)	66	16	1	1	16	46	33	18
Muskrat River										
Litter traps										
Trap 1	1980–81	142 (22)	27	27	42	4	1	2	97	0
	1981–82	169 (33)	27	27	24	1	22	7	91	2
Trap 2	1980–81	8 (4)	7	59	29	2	4	0	100	0
	1981–82	8 (5)	0	71	21	4	3	—	—	—
Trap 3	1980–81	10 (3)	78	2	14	2	4	35	64	1
	1981–82	3 (1)	9	24	33	12	22	0	46	54
Trap 4	1980–81	160 (41)	41	40	14	4	1	4	96	0
	1981–82	121 (20)	56	14	19	1	11	3	52	1
Lateral traps	1980–81	38 (12)	23	25	12	1	40	22	76	1
	1981–82	25 (6)	43	23	6	1	26	7	85	5
Matamek River										
Litter traps										
Trap 1	1980–81	49 (8)	58	5	34	2	1	29	28	1
	1981–82	50 (8)	61	1	16	6	15	61	28	4
Trap 2	1980–81	4 (1)	22	11	16	1	50	38	37	25
	1981–82	17 (11)	58	1	15	3	24	34	65	1
Trap 3	1980–81	2 (1)	89	0	4	0	7	9	91	0
	1981–82	3 (1)	34	1	14	3	48	100	0	0
Trap 4	1980–81	1 (0)	5	1	43	45	6	21	78	0
	1981–82	3 (1)	16	1	26	39	18	33	38	23
Trap 5	1980–81	98 (15)	37	15	40	6	1	29	66	1
	1981–82	62 (16)	46	4	23	16	11	52	46	1
Lateral traps	1980–81	70 (41)	27	36	5	0	32	53	45	1
	1981–82	46 (5)	63	14	10	1	11	43	52	4

litterfall studies (e.g. Bray and Gorham 1964; Gosz et al. 1972), input was highly variable; standard deviations of the mean at these sites were typically 25–75%. The distribution of input over replicate traps was positively skewed for almost all the sampling intervals, indicating a majority of relatively low inputs with occasional high values.

Differences in composition of litterfall between the terrestrial and headwater sites reflect the development of a distinct riparian zone. Both forest plots, although different in density and species composition, had litterfall composed of roughly 50% deciduous leaves, 25% needles, and 10–15% fine woody debris (Table 1). Streamside traps at First Choice Creek collected more leaves and wood than did the nearby forest traps but still received 13–18% of total income as needles. Overall, the composition

and pattern of litter inputs to this narrow first-order stream closely resembled those to the adjacent forest floor (Fig. 2; Table 1). The development of a distinct alder-dominated riparian zone along the lower reaches of Beaver Creek, however, resulted in litterfall very different from that in the adjacent terrestrial site. The stream site received only 2–5% of its total income as needles and less than 10% as wood. The stream site also received less birch leaf input than did the forest plot, instead of collecting 26–46% of its income as alder leaves. At the upstream pond site on Beaver Creek the meadow area acted as a physical separator between stream and forest. The traps at this site also collected much smaller percentages of wood and conifer needles and a larger proportion of alder leaves than in forest litterfall (Table 1).

In addition to changes in composition, a progressive decline in maximum input can be seen from First Choice Creek to the Beaver Creek pond site (Fig. 2). The height of the 1980 autumnal maximum input was 21.6 g AFDM·m^{-2}·d^{-1} at First Choice Creek, 17.1 g AFDM·m^{-2}·d^{-1} at the closed canopy portion of Beaver Creek, but only 7.1 g AFDM·m^{-2}·d^{-1} at the beaver pond site. The development of physically and vegetationally distinct riparian zones on these small streams is accompanied by changes in both the magnitude and the composition of litterfall. This result agrees with the findings of Hill (1981), Webster (1977), and Cummins (1974), who showed decreases in particulate allochthonous input associated with more open riparian vegetation in North Carolina and Michigan. These studies support the assertion of Cummins (1980) that the physical and vegetational structure of the riparian zone is an important factor controlling small stream processes.

Data from these sites also illustrate the disproportionate contribution of deciduous trees to litterfall. Even though greater than 60% of the basal area in each forest plot was spruce or fir trees, less than 30% of the litter collected at the terrestrial sites was conifer needles. Autumn leaf fall has the strongest effect on seasonal pattern; 64–78% of the total annual income to the small stream and terrestrial sites for 1980–81 was collected between September 1 and November 1. For 1981–82, 59–82% of the annual income was collected during this period, although longer sampling intervals and more gradual leaf abscission resulted in a less sharply defined input pattern (Fig. 2). The annual production cycle of deciduous leaves, as opposed to the 5- to 6-yr turnover time for spruce needles in boreal forests (Rodin and Bazilevich 1967), makes the deciduous portion of the forests the dominant source of allochthonous input mass at our study sites (Table 1).

Direct input — Muskrat and Matamek rivers

Litter input to the array traps on the Muskrat and Matamek rivers was collected from May 1980 through November 1981. Mean input values for these rivers were much smaller than at the headwater sites (Fig. 3). The trap positions closest to shore in each river collected less than 0.5 g AFDM·m^{-2}·d^{-1} for most of the year, with seasonal maxima less than 4.0 g AFDM·m^{-2}·d^{-1}. Input to midchannel traps at both sites rarely exceeded 0.1 g AFDM·m^{-2}·d^{-1}, and traps in these positions were frequently empty. Standard deviations were typically 80–120% of the means due to spatial variability of input and small sample size. Because of the low input rates, seasonal patterns were easily obscured by random inputs (Fig. 3); only 44–55% of annual direct input occurred between September 1 and November 1 of each year.

The small input mass also resulted in irregular compositional data for these sites (Table 1), but in general, composition reflected local riparian structure as at the headwater sites. The Muskrat River received the highest proportions of needle and alder input, reflecting the relative absence of birch and aspen trees in the vicinity. The Matamek River traps received a mixed input of birch leaves, alder leaves, and needles. At neither site were there consistent differences in composition between nearshore and midchannel traps.

Lateral input — all sites

Lateral input at each of the study sites was sampled from September 1980 through May 1982. As with direct inputs to the river array traps, the mass of material collected by individual traps was small and the variability high. Standard deviations of mean input ranged from 100 to 200%, and distributions were strongly positively skewed. Mean inputs ranged from late summer values of 0–00.3 g AFDM·m^{-1}·d^{-1} to peaks of 0.29–1.81 g AFDM·m^{-1}·d^{-1} in autumn.

Seasonal patterns similar to those seen in direct fall were observed at First Choice Creek and at the downstream closed canopy site on Beaver Creek, but at other sites input was irregular (Fig. 4). A consistent feature at all sites, however, is proportionally higher input during the first overwinter period. In the direct-fall traps, and in the lateral traps during the second study year, overwinter input was less than 20% of total annual income. Collections of overwinter lateral input during the first study year, however, included up to 80% of annual income. This difference is best explained by the timing of our sampling dates relative to spring runoff. In 1981, snowmelt and peak discharge occurred before sampling of the lateral traps. Several traps at all sites were lost or overturned in floodwaters. Effects of the flooding were most severe at the Beaver Creek open canopy site and on the Matamek River. In 1982, traps were collected before peak discharge, while snow was still on the ground. These second-year collections reflect winter inputs and are similar in relative magnitude to winter inputs in direct fall. The large amount of wood and other debris in the first-year collections probably represents a sudden input of material entrained by retreating floodwaters rather than the high overwinter input implied by the figure.

There is also a noticeable difference in composition between the lateral inputs and the corresponding direct input at each study site (Table 1). At every stream site, lateral traps collected smaller proportions of needle and catkin input than did litter traps. This difference was also seen by Triska and Cromack (1980), who reported that only 29% of needles collected in Watershed 10, Oregon, came from lateral transport compared with 58% for leaves. McDowell and Fisher (1976) reported that hemlock needles contributed 13% of the autumn litterfall to Roaring Brook, Massachusetts, but only 3% of the corresponding lateral input. Loss of needles through the 5-mm mesh of the lateral traps may be responsible for some of the reduction in inputs seen in this study, but the low surface-to-volume ratio of the needle and catkin fractions also makes them less susceptible to wind-driven transport, the dominant mechanism of lateral movement in the Matamek region.

Annual Estimates of Particulate Allochthonous Input

Annual estimates of particulate input ranged from 534 g AFDM·m^{-2}·yr^{-1} at First Choice Creek to 2 g AFDM·m^{-2}·yr^{-1} for midchannel traps on the Matamek River (Table 1). Annual litterfall at the terrestrial sites was lower than has been reported

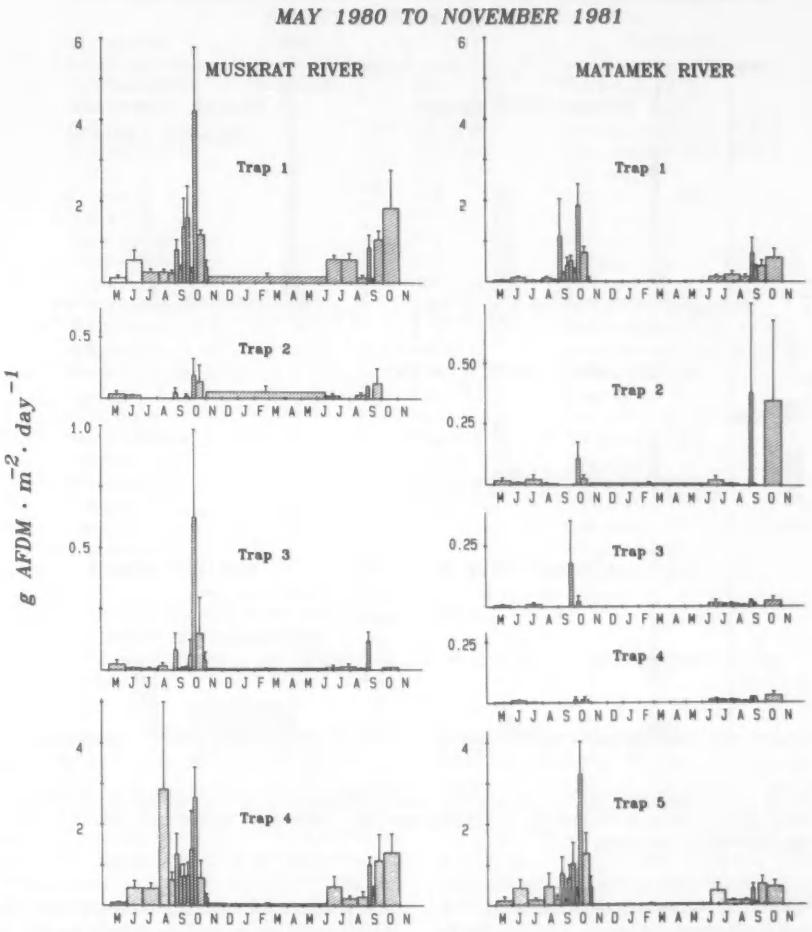
ALLOCHTHONOUS INPUT - ARRAY TRAPS
MAY 1980 TO NOVEMBER 1981
MUSKRAT RIVER
MATAMEK RIVER
Trap 1
Trap 2
Trap 3
Trap 4
Trap 5
M J J A S O N D J F M A M J J A S O N
g AFDM · m⁻² · day⁻¹

FIG. 3. Allochthonous input to the Muskrat and Matamek rivers array traps. Each histogram cell represents one sampling interval: width indicates span of the interval; height indicates mean input to the numbered trap position from 5 replicate transects (see Fig. 1, text). Bars show standard errors of the mean.

for temperate forests (Gosz et al. 1972; Bell et al. 1978; Bray and Gorham 1964) but was similar to that reported for New Brunswick pine and deciduous forests (MacLean and Wein 1978) and for spruce forests in the Soviet Union (Rodin and Bazilevich 1967). The high latitude and short growing season in subarctic eastern Quebec result in lower forest productivity and less litterfall than in temperate regions where most other allochthonous input studies have been conducted (Table 2).

Standard errors associated with annual input estimates were surprisingly low, considering the small sample size at each study site (Table 1). Errors were <10% for the terrestrial sites and <15% for all but one of the headwater stream sites. Standard errors for lateral input estimates were 18–59% during the first year due to high variances and lost traps in the overwinter interval, but were only 8–24% during the second study year.

Litterfall versus Distance from Shore

It is clear from Fig. 3 and Table 1 that litterfall to the Muskrat and Matamek rivers follows a pattern of maximum input near the shorelines and decreased input in midstream. This observation is consistent with the findings of previous authors (Fisher 1977; Matthews and Kowalczewski 1969) and with intuitive assumptions about the role of allochthonous input in large streams (Cummins 1977). The problem remains, however, to find a quantitative description of this trend that leads to realistic estimates of overall inputs to these streams, without overestimating the importance of bank litterfall or underestimating the large collecting surface of the open channel.

Figures 5 and 6 show annual input estimates for each of the individual litter traps in the Muskrat and Matamek rivers plotted against distance from shore. These figures demonstrate the

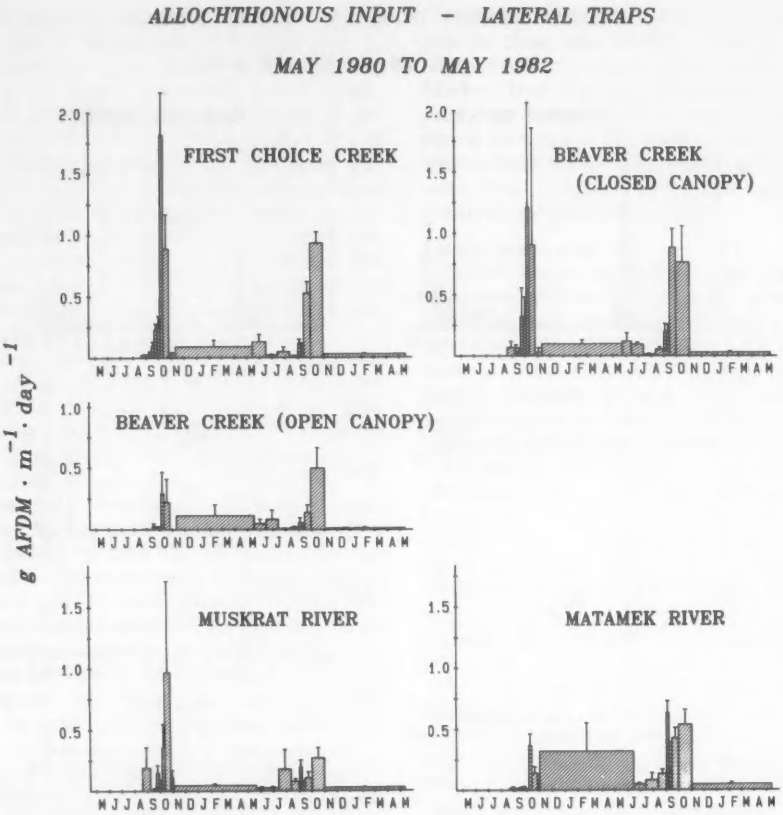
ALLOCHTHONOUS INPUT - LATERAL TRAPS
MAY 1980 TO MAY 1982
FIRST CHOICE CREEK
BEAVER CREEK (CLOSED CANOPY)
BEAVER CREEK (OPEN CANOPY)
MUSKRAT RIVER
MATAMEK RIVER
g AFDM · m⁻¹ · day⁻¹
M J J A S O N D J F M A M J J A S O N D J F M A M

FIG. 4. Allochthonous input to the lateral traps. Each histogram cell represents one sampling interval: width indicates span of the interval; height indicates mean input to 6–10 replicate traps. Bars show standard errors of the mean.

extreme spatial variability of particulate input at these sites. When regressions were performed, however, 71–73% of the variation observed on the Muskrat River and 46–65% of that on the Matamek River was accounted for by a simple exponential model (see figure legends for equations). This model gave the best fit to our data, as it did with Gasith and Hasler's (1976) replot of Sczepanski's (1965) data from Lake Mikolajki, Poland. A linear decay model was used by Matthews and Kowalczewski (1969) on the Thames River and by Gasith and Hasler (1976) on their data from Lake Wingra, Wisconsin. With our data, linear decay accounted for only 50–65% and 36–60% of the variation in the Muskrat and Matamek rivers, respectively. Integration of the exponential regression curve gave annual input estimates of 37 and 30 g AFDM·m^{-2} for the two years, respectively, for the Muskrat River and 15 and 17 g AFDM·m^{-2}, respectively, for the Matamek River. These integrated values were used when examining trends in input with stream order.

Trends in Allochthonous Input with Stream Order

Direct litterfall

The most striking feature of the annual input estimates in Table 3 is the progressive decline in direct litterfall with increasing stream order. When annual litter input for each study year is regressed against stream order for First Choice Creek, the Closed Canopy site on Beaver Creek, the Muskrat River, and the Matamek River, the resulting exponential declines have regression coefficients >0.99 (Fig. 7). When data from both years are combined, the regression curve falls between the individual year curves with $r^2 = 0.98$. This decline provides strong quantitative support for the stream continuum models of Vannote et al. (1980) and Cummins (1977). It implies that allochthonous energy per unit of stream area becomes progressively less in larger streams, shifting stream trophic structure away from the detritus-based economy of headwater streams. The rate of this shift in different watersheds might be expected to differ from that for the Matamek River basin, but the regularity of the change with stream order is probably a general property for all forested watersheds.

An interesting feature of these regressions is that the direct inputs to the Beaver Creek Open Canopy site fall along the curve at the level of a third-order stream for both years. This suggests that the dam building and browsing activities of beaver effect riparian and channel structure sufficiently to alter stream properties, increasing the functional order of the stream in ponded areas.

Lateral input

There was no trend in lateral input with stream order. The

TABLE 2. Annual litterfall as reported in this study and in the literature.

Location	Stream order	Litter input ($g \cdot m^{-2} \cdot yr^{-1}$)	Reference
New Hampshire	Terrestrial	570	Gosz et al. 1972
Illinois		520–629	Bell et al. 1978
Ontario		450–818	Merriam et al. 1982
New Brunswick		152–197	MacLean and Wein 1978
Quebec		229–327[a]	This study
Denmark	1	655[a]	Iversen et al. 1982
North Carolina		353	Webster 1977
North Carolina		319	Webster 1977
North Carolina		285	Webster 1977
Quebec		307–534[a]	This study
New Hampshire	2	578[a]	Fisher and Likens 1973
Massachusetts		274[b]	McDowell and Fisher 1976
Mississippi		400	de la Cruz and Post 1977
North Carolina		273	Webster 1977
Quebec		212–312[a]	This study
Mississippi	3	386	Post and de la Cruz 1977
North Carolina		572–638	Mulholland 1981
Quebec[c]		103–120[a]	This study
Massachusetts	4	384	Fisher 1977
Quebec	5	30–37[a]	This study
Quebec	6	15–17[a]	This study
Thames River, England		18–28	Matthews and Kowalczewski 1969

[a]Input is in g AFDW; all others are in dry weight.
[b]Includes only autumnal input.
[c]From the Beaver Creek Open Canopy site (see discussion on litter input versus stream order).

Beaver Creek Open Canopy and Muskrat River sites received less lateral income than the other sites, but this difference is best explained on the basis of localized differences in riparian vegetation. The forest bordering the Muskrat River had a higher proportion of coniferous trees than any of the other sites, and the reduced representation of needles in lateral transport was discussed earlier. At the Open Canopy site on Beaver Creek, the sedimented area on either side of the stream channel acts as an obstacle to lateral transport of forest litter. At First Choice Creek, the Beaver Creek Closed Canopy site, and the Matamek River, wind-driven transport of nearby forest and riparian leaf litter appeared independent of channel structure and stream size.

The role of laterally transported litter in total allochthonous income, however, was strongly influenced by stream width. First Choice Creek is so narrow that lateral inputs make up close to half of the total litter income (Table 3). As the collecting surface of the stream increases, direct litterfall grows to be much larger than the constant lateral supply, until lateral input contributes only 7% of the total at the Muskrat River and 10% at the Matamek River.

Fisher (1977) suggested that the slope of the stream banks was the controlling factor in determining the role of lateral inputs. This factor clearly accounts for some of the differences between central Massachusetts and Watershed 10 in Oregon. In the Matamek region, however, all of the streams are low gradient and stream bank slopes slight. In this low-gradient region the importance of lateral input is indeed smaller in large streams than in the headwaters, but the controlling factor appears to be stream width rather than valley slope.

Total input per length of stream

Estimates of direct and lateral input rates may be combined with the dimensions of the stream channel to calculate total allochthonous loading to each unit length of stream (Table 3). Total input per metre at our study sites increased approximately logarithmically with stream order, from 200–272 g AFDM$\cdot m^{-1} \cdot yr^{-1}$ at First Choice Creek to 928–986 g AFDM$\cdot m^{-1} \cdot yr^{-1}$ at the Matamek River. This trend runs opposite to the decline in areal input and illustrates an important distinction between the consideration of allochthonous input as a food resource and as a mass-balance input. Because the input rate per unit area is low, large particulate detritus is not usually the primary food supply in large streams (Matthews and Kowalczewski 1969; Penczak et al. 1976; Fisher 1977). Authors concerned with trophic dynamics of stream communities therefore tend to consider particulate allochthonous input negligible in these streams. For consideration of mass and nutrient transfer from the terrestrial to the aquatic ecosystem, however, it is the total allochthonous input that is important. While particulate allochthonous inputs to large streams may not be a major factor in local metabolism, their contribution to total mass inputs for the drainage network may be significant.

Watershed Loading Estimates

Geomorphological data on the Matamek River drainage network were combined with the regressions of Fig. 7 to estimate annual particulate allochthonous loading to the drainage network (Table 4). The results give a quantitative example of the relative contribution of different stream sizes to watershed income. The headwater streams (orders 1–3) each contributed approximately 22% of total loading. In these orders the increase in total allochthonous input per unit length is balanced by the decreased number of streams of higher orders. For larger

TABLE 3. Annual litterfall, lateral input, and total particulate input. The litter inputs for the Muskrat River and Matamek River are estimates from the regressions in Fig. 5 and 6. See methods for explanation of shore/stream ratios and total input calculations.

Site	Stream order	Study year	Litter traps (g AFDM·m^{-2}·yr^{-1})	Lateral traps (g AFDM·m^{-1}·yr^{-1})	Mean width (m)	Shore/ stream	Total input (g AFDM·m^{-1}·yr^{-1})	% of total as lateral input
First Choice Creek	1	1980–81	534	53	0.3	2.11	272	41.1
		1981–82	307	51			200	53.9
Beaver Creek								
Closed Canopy	2	1980–81	312	53	2.0	2.20	741	15.7
		1981–82	212	55			545	22.2
Open Canopy		1980–81	120	30	3.4	2.26	476	14.2
		1981–82	103	26			409	14.4
Muskrat River	5	1980–81	37	38	21.9	2.10	890	9.0
		1981–82	30	25			709	7.4
Matamek River	6	1980–81	15	70	51.7	2.32	938	17.3
		1981–82	17	46			986	10.8

streams (orders 4–6) these calculations show the strongest effect of increases in input per unit length. Even though the largest streams in this watershed make up only 15% of the total stream length, they have 73% of total stream surface area and collect 30% of the total allochthonous input. Inputs to larger streams in the watershed may not be especially important in local metabolism, but they receive a significant proportion of the carbon and nutrient export from the terrestrial system to the drainage network.

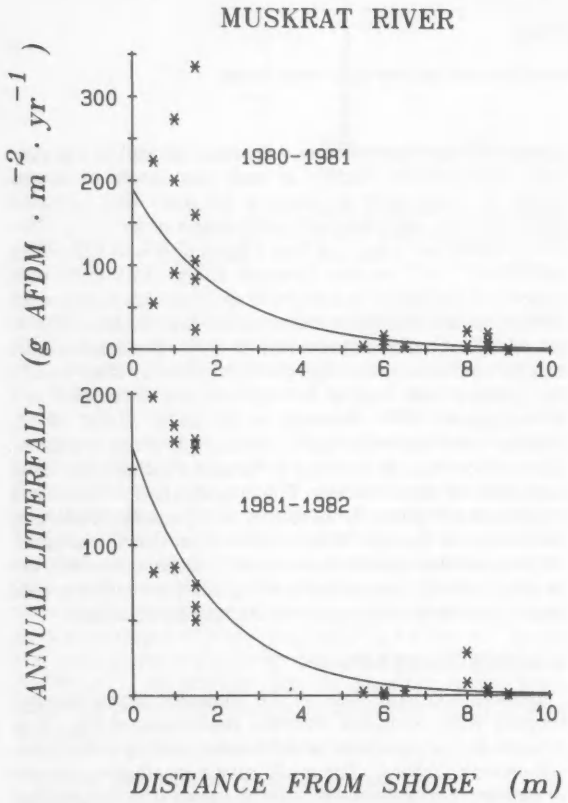

FIG. 5. Annual allochthonous input to the Muskrat River as a function of distance from shore. The exponential regressions are $y = 190e^{-0.459x}$ ($r^2 = 0.71$) for 1980–81 and $y = 164e^{-0.506x}$ ($r^2 = 0.73$) for 1981–82.

Allochthonous Inputs and Autochthonous Production

Studies of allochthonous input to stream ecosystems are ultimately concerned with the role of that input in stream metabolism, either with the relative contributions of allochthonous and autochthonous sources to the total energy available, or with the autotrophic or heterotrophic state of a stream segment as measured by mass balance or production to respiration (P/R) ratios. Naiman (1983a) studied the oxygen metabolism of the four streams in our study and estimated gross primary productivity and P/R ratios. In accordance with the predictions of current conceptual stream models (Cummins 1977; Vannote et al. 1980; Minshall et al. 1983), first-order First Choice Creek and second-order Beaver Creek (the Closed Canopy region) had annual P/R ratios less than 1, while annual ratios for the fifth-order Muskrat River and sixth-order Matamek River were greater than 1. Table 5 compares the gross photosynthesis of the periphyton and macrophyte communities in these streams with the 2-yr averages of particulate allochthonous input. This table does not yet include the important dissolved and fine particulate organic matter fractions, but the data from the two sources

TABLE 4. Particulate allochthonous loading to the Matamek River watershed. See text for calculation methods. Geomorphological data are from Naiman (1983a, 1983b).

	Watershed composition		Allochthonous income	
Stream order	Total length (km)	% of total length	Loading (10^3 kg AFDM)	% of total mass
1	726	50.5	165.2	22.7
2	322	22.4	179.8	24.7
3	176	12.2	159.3	21.9
4	92	6.4	98.4	13.5
5	95	6.6	100.7	13.8
6	26	1.8	25.4	3.5
Total	1437		728.6	

TABLE 5. Allochthonous input versus autochthonous production. The average of annual particulate allochthonous inputs from the 2 yr of this study is compared with estimates of primary production from Naiman (1983a). Original units are converted to grams carbon according to de la Cruz and Post (1977): 1 g O_2 = 0.375 g C, 1 g AFDM = 0.45 g C.

Stream	Carbon supply ($g\,C \cdot m^{-2} \cdot yr^{-1}$)			% of total	
	Primary production	Allochthonous input[a]	Production + input	Primary production	Allochthonous input[a]
First Choice Creek	20.51	354.00	374.51	5.5	94.5
Beaver Creek	34.35	144.68	179.03	19.2	80.8
Muskrat River	96.49	16.43	112.92	85.4	14.6
Matamek River	168.09	8.37	176.46	95.3	4.7

[a]Does not include carbon supplied as DOM or POM in suspension.

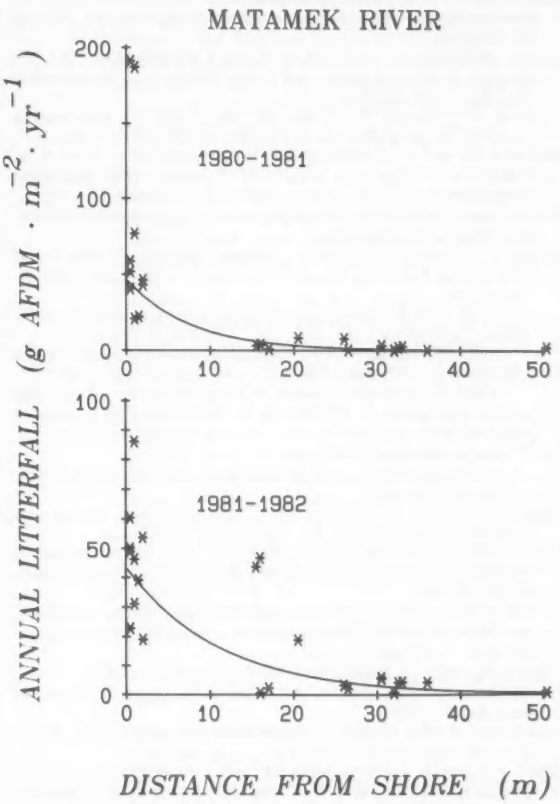

FIG. 6. Annual allochthonous input to the Matamek River as a function of distance from shore. The exponential regressions are $y = 46.6e^{-0.113x}$ ($r^2 = 0.46$) for 1980–81 and $y = 43.4e^{-0.089x}$ ($r^2 = 0.65$) for 1981–82.

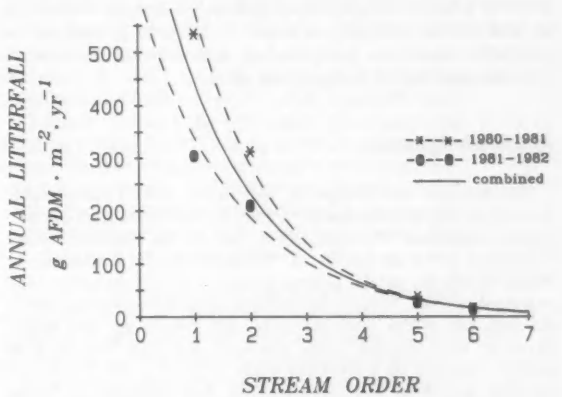

FIG. 7. Annual litter input (direct fall) as a function of stream order for First Choice Creek, Beaver Creek (Closed Canopy site), the Muskrat River, and the Matamek River. Broken lines indicate exponential regressions for data points from the individual years: $y = 1191e^{-0.713x}$ ($r^2 = 0.99$) for 1980–81 and $y = 616e^{-0.598x}$ ($r^2 = 0.99$) for 1981–82. The solid line indicates the regression using data from both years: $y = 856e^{-0.598x}$ ($r^2 = 0.98$).

shown indicate a smooth, complete transition from allochthonous carbon supply in the headwater streams to autochthonous production in the midsized rivers.

The River Continuum Concept and Riparian Control of Allochthonous Inputs

The River Continuum Concept (Vannote et al. 1980) asserts that progressive changes in the balance of stream processes are produced by changes in kinetic energy dissipation patterns along the physical gradient from headwaters (stream orders 1–3) to large rivers (orders >6). One shift expected in response to these changes is a decline in the importance of terrestrial organic input. Minshall et al. (1983) stated as an explicit assumption in the formation of their hypotheses "If the relative contribution of organic matter from the riparian zone decreases downstream ..." One of the objectives of this study was to quantitatively test this assumption in a pristine forested system.

Our results strongly support this common assumption, as well as providing preliminary evidence in support of expected P/R ratios and metabolic shifts along the size gradient. In addition to continuum trends, however, many of our results reflected the influence of local factors. Composition of litter inputs and lateral input rates, in particular, were more strongly determined by local physical and vegetational riparian structure than by stream size.

This dependence of input characteristics on local riparian structure illustrates the information loss inherent in a description of end effects such as the River Continuum Concept. While this model is an effective generalization for organizing our understanding of stream processes in most watersheds, it is the

product of a synthetic rather than a mechanistic approach. In our study area, for example, the combined effects of physical, vegetational, and litterfall characteristics produced a declining downstream trend in direct litterfall. This pattern is common in forested watersheds where streamside vegetation becomes more open as riparian zones develop in association with larger streams. To make predictive statements about allochthonous input in nonforested or disturbed watersheds, however, we need to understand the process independently of a priori generalizations about riparian vegetation and stream order. Breaking the complex process into site-independent components, we need to know (1) what physical changes in channel and valley structure are associated with geomorphological definition of the drainage network, (2) how these changes in elevation and gradient interact with climate and soils to determine riparian vegetational structure, and (3) the character of allochthonous inputs produced by the riparian types thus created. When these factors can be understood separately we will be able to predict, on a watershed scale, the total loading and seasonal patterns of allochthonous inputs to any given system.

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Efficiency Estimates for Various Quadrat Sizes Used in Benthic Sampling

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A review of some of the marine littoral ecological literature revealed that area sampling is frequently used to assess biomass and both species densities and associations. The three most common sample unit configurations were the quadrat, circle, and rectangle; their popularity was 66.7, 19.0, and 14.3%, respectively. The percentage of researchers using areas of <0.25, 0.25, 1.0, and >1.0 m^2 was 42.9, 23.8, 28.6, and 4.8%, respectively; their reasons for using these areas were not stated. My sampling study was designed to determine the efficiency of six sample unit sizes (0.25, 1.0, 1.56, 2.25, 2.99, and 4.0 m^2) when assessing macrophyte biomass. The cost in time to harvest a fixed sample of 20 m^2 varied markedly with sample unit size, as did sampling precision. The rates to harvest 1 m^2 with the 2.25- and 0.25-m^2 quadrats were 9.1 and 34.8 min, respectively; the respective sampling precisions (CV) were 0.176 and 0.075. The formula $n = [S/(\bar{x} \cdot CV)]^2$ was used to determine sample unit number for a given precision of the estimate (CV's = 0.075, 0.10, and 0.15). The cost in time to attain a sampling precision of 0.10 for the 0.25- and 2.25-m^2 sampling units was estimated at 393.2 and 569.9 min, respectively. A large number of small sample units (0.25 m^2) was more efficient than a smaller number of large sample units (4.0 m^2). I recommend that sampling efficiency be determined prior to the initiation of an area sampling program.

L'examen d'une partie des communications sur l'écologie des milieux marins et littoraux a montré que l'échantillonnage basé sur la superficie était fréquemment utilisé pour évaluer la biomasse ainsi que la densité et les associations des espèces. Les trois configurations unitaires d'échantillonnage les plus courantes étaient le quadrat, le cercle et le rectangle dont les fréquences respectives d'utilisation étaient : 66,7, 19,0 et 14,3 %. Les pourcentages de chercheurs utilisant les superficies <0,25, 0,25, 1,0 et >1,0 m^2 étaient respectivement de 42,9, 23,8, 28,6 et 4,8 % ; les raisons du choix d'une superficie donnée n'étaient pas mentionnées. Mon étude de l'échantillonnage était conçue de façon à déterminer l'efficacité de six surfaces unitaires de prélèvement (0,25, 1,0, 1,56, 2,25, 2,99 et 4,0 m^2) pour l'évaluation de la biomasse de macrophytes. Le temps et la précision de l'échantillonnage pour le prélèvement d'un échantillon constant de 20 m^2 variaient de façon appréciable en fonction de la taille unitaire de l'échantillon. Le temps nécessaire à l'échantillonnage de 1 m^2 en utilisant des quadrats de 2,25 et 0,25 m^2 était respectivement de 9,1 et 34,8 min et les précisions d'échantillonnage (CV) respectives de 0,176 et 0,075. La formule $n = [S/(\bar{x} \cdot CV)]^2$ a été utilisée pour déterminer le nombre d'unités d'échantillonnage correspondant à une précision donnée de l'estimation (CV = 0,075, 0,10 et 0,15). Les durées nécessaires à l'obtention d'une précision d'échantillonnage de 0,10 avec les unités d'échantillonnage de 0,25 et 2,25 m^2 ont été estimées à 393,2 et 569,9 min respectivement. Un nombre élevé de petites unités d'échantillonnage (0,25 m^2) s'avérait plus efficace qu'un nombre plus restreint d'unités plus grandes (4,0 m^2). Il est recommandé de déterminer l'efficacité de l'échantillonnage avant de mettre sur pied un programme d'échantillonnage par unité de surfaces.

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Evaluations of techniques for quantitative sampling of shallow epibenthos, including macrophytes, are rare. Holme and McIntyre's (1971) handbook was principally for "...sampling of sediments and their fauna...". Round and Hickman's (1971) dealt mainly with techniques for estimating primary productivity. Schwenke (1972) correctly concluded that a generally accepted technology for sampling benthic macrophytes does not exist.

I reviewed 21 papers (Table 1) involved with either determining density, biomass, or species associations in the shallow sublittoral or eulittoral zones. The other criterion for their inclusion in the survey was that *area sampling* ("...methods in which the sampling unit is an area of land" (Snedecor and Cochran 1956)) techniques were employed. The most commonly used sample unit shape (66.7%) was the quadrat, "...a square of varying size..." (Clements 1907); 19% employed circles and 14.3% used rectangles. The workers did not give reasons why particular sample unit shapes or sizes were chosen.

The quadrat size employed is important (Clapham 1932; Greig-Smith 1952; Wiegert 1962). First, the conclusions reached on the distribution of the target species or degree of association between species is a function of quadrat size (Elliot 1971; Kershaw 1973). Second, because the cost of sampling for a given precision in the terrestrial habitat has been shown to vary markedly with quadrat size (Wiegert 1962), this should also be important in the marine environment. Snedecor and Cochran (1956) pointed out that an investigator should choose that sample unit size that will yield the greatest precision for

TABLE 1. Survey of sampling units employed by benthic marine biologists.[a]

	Area (m^2)				
	<0.25	0.25	1.0	>1.0	Total
Quadrats	3	4	6	1	14
Circles	3	1	0	0	4
Rectangle	3	0	0	0	3
Total	9	5	6	1	21

[a]Sources: Aleem 1956; Baardseth 1970; Caplan and Boolootian 1967; Doty 1971; Fowler and Fletcher 1975; Himmelman 1980; Hines 1982; Hulbert et al. 1982; Lewis et al. 1981; Littler 1971, 1980; Littler and Murray 1975; Mathieson et al. 1977; Mathieson et al. 1981; McPeak 1980; Nicholson and Cimberg 1971; Pielou 1981; Pringle and Semple 1980; Sammarco 1982; Taylor 1971.

available resources. If the sample size is fixed, e.g. 5% of the total area, a large sampling unit will usually give less accuracy than a small sampling unit. However, this is frequently counterbalanced by an inverse relationship between size of the sample unit and cost in sampling time.

Biomass estimates of the commercially important seaweed *Chondrus crispus* Stackhouse (Irish moss) were required. The coastal marine environment is frequently a hostile habitat in which to undertake ecological studies (Schwenke 1972). As well, monetary costs for sampling are high due to the need for ancillary equipment such as boats and SCUBA. Consequently, my study was undertaken to determine both the sampling efficiency and precision of a logical size-range of quadrat-shaped sampling units.

Methods and Materials

The sample unit sizes ranged from 0.25 to 4.0 m^2. They were constructed of tubular steel rod (approx. 7.0 m in diameter) welded at the joints. The Pleasant View Irish moss bed (46°54′38″N; 64°12′26″W) off western Prince Edward Island (for precise location and description of the bed see Pringle and Semple 1983) was the study site. Six 0.5-ha squares, haphazardly chosen, were demarcated by buoys. The biomass of interest was that vulnerable to commercial dragrakes, i.e. the harvestable biomass. Consequently, SCUBA-equipped divers used handrakes with dragrake tine spacings (approx. 6.5 mm) (Pringle et al. 1981) for frond removal. Each sample unit was chosen haphazardly by dropping the quadrat from the diving tender. The fronds were raked from the sample unit and placed in a fine-mesh sample bag while another quadrat was dropped. The quadrat and sample unit were transported to the tender by rope while the sampler moved to the next sample unit. The harvesting time for each sample unit was recorded for all quadrat sizes except the 1.0 m^2; for the latter, total time was, however, noted.

The contents of each bag were transferred to a polyethylene bag in the tender and transported to the laboratory where the fresh weight was recorded; the sample was then frozen at −30°C. Within 30 d, all sample units had been thawed and *C. crispus* was separated from the other macrophytes (by catch) and its fresh weight recorded.

The two variables I employed to compare the quadrats were time and precision of the estimate (coefficient of variation, with the mean being the biomass per sample unit). To permit comparisons between quadrats, I planned to use a fixed sample base. Because of slight variations in the latter, however, I corrected for these as shown in Table 2. Biomass per sample varied markedly (Table 2); this was corrected by standardizing biomass to that sample with the largest biomass (3.0-m^2 quadrat; 36 252.0 g) as follows:

$$SF = 36\,252.0/B$$

where SF = standardizing factor and B = biomass of the respective sample. The SF was then used to correct the mean time ($\bar{t}$) to harvest each sample unit as follows (Table 2):

$$\bar{t}c = SF \cdot \bar{t}$$

where $\bar{t}c$ = corrected mean time. This correction assumes a linear relationship between time and biomass for each sample unit size.

To determine the number of sample units (n) required to attain a certain precision (CV of 0.075, 0.10, and 0.15) for each sample unit size, the following formula was employed:

$$CV = \frac{\sigma/\sqrt{n}}{\mu}$$

where

$$n = \left(\frac{\sigma}{\mu \cdot CV}\right)^2 = \left(\frac{S}{\bar{x} \cdot CV}\right)^2.$$

The total time (T) to harvest n sample units was determined as follows:

$$T = n \cdot \bar{t}c.$$

Results and Discussion

If one were concerned with time only, when assessing macrophyte biomass on commercially important Irish moss beds, then a 2.25-m^2 sample unit would be used. With the latter it took 182.5 min to harvest a standardized biomass from a 20-m^2 area (Table 2). The percent savings in time using this sample unit size, compared with the other quadrat sizes, ranged from 12.9% (4.0 m^2) to 74.8% (0.25 m^2). Sampling precision is generally of concern, as well as cost in time, however. The CV for the various sample unit sizes ranged from 0.075 (0.25 m^2) to 0.334 (4.0 m^2) (Table 3).

A linear relationship existed between sample unit number and sample unit size at all three levels of sampling precision (Fig. 1), if the 4.0-m^2 quadrat data were deleted (Table 3). Justification for the latter is based on the unexpectedly large number (99) of 4.0-m^2 quadrat sample units predicted to attain a precision of estimate of 0.075. This is 20 more than was observed for the 0.25-m^2 sample unit. The large standard deviation of the sample (coefficient of variation of the standard deviation was 0.75 vs. 0.40 for the 2.99-m^2 quadrat) is, no doubt, responsible (Table 3). This was because the formula used to determine the number of sample units constituting a sample had the standard error as a function of the square root of n. Consequently, it takes a large increase in n to bring about a small improvement in the standard deviation (Southwood 1968). Thus, the relationship between sample unit number and sampling precision was determined through extrapolation of the regression lines (Fig. 1). This is probably why the estimates were large for the sample unit numbers for the 1.0-m^2 quadrat size as well (Table 3). To avoid this problem when using this technique, one should employ a sample size that will yield a low CV value.

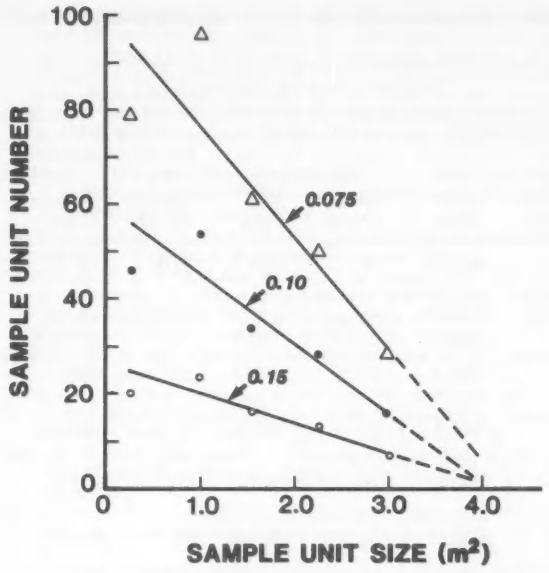

FIG. 1. Relationship between sample unit number, sample unit size (quadrats 0.25–4.0 m^2), and sampling precision (CV of 0.075, 0.10, and 0.15) when assessing *C. crispus* standing crop. The lines for each CV were fitted by eye; broken lines were extrapolated.

The relationship between total sampling time, sample unit size, and sampling precision is curvilinear (Fig. 2). The most inefficient sample unit size, if the large standard deviation is not considered, is that of 1.0 m^2 (Table 3). However, if the sample unit number is reduced, as shown in Table 3, then the sample unit size of 1.56 m^2 is the most inefficient (Fig. 2). The most efficient sample unit size at all levels of sampling precision was the 0.25 m^2. By employing the latter quadrat size the savings in time ranged from 233.4 to 57 min depending on sampling precision required (Table 3).

The accepted level of error varies with the study, but for many purposes an error of 10% is acceptable (Southwood 1968). If one arbitrarily chose a sample unit size of 1.56 m^2 at this sampling precision when assessing macrophyte biomass in the present study area, then the program would cost about 120.5% more in time than if a 0.25-m^2 quadrat were used (Table 3; Fig. 2).

These results confirm Snedecor and Cochran's (1956) observation that, for a fixed sample size, a large sampling unit will generally be less efficient than a small one; however, the former will be less costly in time. There is little doubt that in my study the smallest sampling unit was the most efficient (least cost per unit of precision). Furthermore, I confirm Pielou's (1974) contention that, prior to the intitiation of a sampling program, a special investigation on sampling procedure should be undertaken to determine the most efficient sampling unit size.

It is unfortunate that a sample unit size smaller than 0.25 m^2 was not part of this experiment. Mathieson and Burns (1975) employed a 0.1-m^2 quadrat when sampling *C. crispus* from New England rocky shores; however, Lilly (1968) and Taylor (1973) used a 1.0-m^2 sample unit when assessing Irish moss in the Gulf of St. Lawrence. Holme (1971) did discriminate between size of quadrats; he recommended that 1.0 m^2 be employed when sampling large benthic animals on a rocky

TABLE 2. Sample time and biomass harvested per sample unit size plus the corrected sample time per standardized sample biomass. Columns are labelled for subsequent reference.[a]

(1) Quadrat dimensions (m)	(2) Sample unit area (m^2)	(3) No. of sample units[b]	(4) Sample area (m^2)	(5) Total time to sample (min)	(6) Mean time/ sample unit (min) (SD)	(7) Sample time corrected for area (min)	Sample biomass (g fresh wt) (8) Uncorrected	(9) Corrected for area	(10) Standardizing factor based on biomass	(11) Corrected time/ sample unit (min)	(12) Corrected time to sample (min)
0.50×0.50	0.25	79(80)	19.75[c]	530	6.7(1.9)	536.6	30 476.3	30 857.3	1.30	8.7	696.0
1.0×1.0	1.0	20	20.0	240	12.0	240.0	26 277.2	26 277.2	1.52	18.2	364.0
1.25×1.25	1.56	13(12.8)	20.3[d]	172	13.2(4.8)	169.4	21 035.0	20 719.5	1.93	25.5	326.4
1.50×1.50	2.25	9(8.9)	20.3[d]	103	11.4(4.9)	101.5	22 592.3	22 253.4	1.8	20.5	182.5
1.73×1.73	2.99	6(6.7)	17.9[e]	198	33.0(15.3)	218.8	36 252.0	40 058.5[f]	1.0	33.0	221.1
2.0×2.0	4.0	5	20.0	115	23.0(12.3)	115.0	21 989.2	21 989.2	1.82	41.9	209.5

[a]Column (7) = column 5 (5) × 20 ÷ column (4); column (9) = column (8) × 20 ÷ column (4); column (10) is from column (8); column (11) = column (6) × column (10); column (12) = column (5) × column (10).
[b]No. in parentheses is sample unit no. required to yield 20 m^2.
[c]Missing sample unit (0.25% of sample).
[d]Sample too large by 1.5%.
[e]Missing 10.5% of sample.
[f]All samples standardized to this biomass.

TABLE 3. Statistics per sample unit size plus the estimated sample unit number and cost in time for three levels of sampling precision (CV of 0.075, 0.10, and 0.15).

(1) Sample unit area (m^2)	Empirical results: Biomass/sample unit area				Estimated sampling efficiency					
					CV of 0.075		CV of 0.10		CV of 0.15	
	(2) Mean (g fresh wt)	(3) SD (CV)	(4) SE	(5) CV	(6) Sample unit no.	(7) Cost (min)	(8) Sample unit no.	(9) Cost (min)	(10) Sample unit no.	(11) Cost (min)
0.25	381.0	255.6 (0.67)	28.6	0.075	79	687.3	46	393.2	20.0	174.0
1.0	1313.9	962.9 (0.73)	215.3	0.164	96[a](70)	1739.9 (1270.4)[b]	54[a](40)	977.3 (728)[c]	24[a](18)	435.0 (327.6)[d]
1.56	1618.1	943.8 (0.58)	261.8	0.162	61	1540.2	34	867.0	16	385.1
2.25	2510.3	1323.0 (0.53)	441.0	0.176	50	1012.7	28	569.9	13	254.2
2.99	6042.0	2393.1 (0.40)	977.0	0.162	28	920.7	16	518.1	7	231.0
4.0	4397.8	3280.9 (0.75)	1467.3	0.334	99	4148.1	56	2333.8	25	1034.9

[a]Sample unit no. for 0.25 m^2 + sample unit no. for 1.56 m^2 ÷ 2.
[b]Column (6) (no. in parentheses) × column (11) from Table 2; a more realistic estimate of total sample time.
[c]Column (8) (no. in parentheses) × column (11) from Table 2; a more realistic estimate of total sample time.
[d]Column (10) (no. in parentheses) × column (11) from Table 2; a more realistic estimate of total sample time.

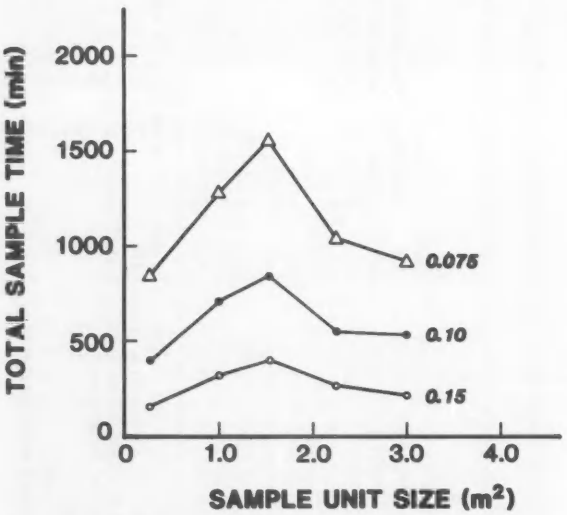

FIG. 2. Relationship between total sample time per sample unit size and sampling precision (CV of 0.075, 0.10, and 0.15) when assessing *C. crispus* standing crop.

shore and 0.25 m^2 for lesser sized animals. Reasons for the choice of any of the above sample units were not given.

Based on these results, I suggest the use of a 0.25 m^2 sampling unit or smaller when sampling benthic organisms that are the size and distribution of the macrophytes (see Pringle and Sharp 1980) sampled here. It is obvious, however, that for most large studies the time invested in assessing sample unit size will be rewarded in a more efficient sampling procedure. This will most certainly be the case where time is of essence, e.g. where boat time is expensive or SCUBA is employed.

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Socioeconomic Aspects of Sea Cage Salmon Farming in the Maritimes

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Commercial salmon farming in the Maritimes shows potential as a source of rural employment, growth, and foreign exchange. Canada lags behind most advanced countries in developing its aquaculture industry; Atlantic salmon (*Salmo salar*) might be one species in which Canada has a comparative advantage and in which Canada's late start might be overcome. This paper evaluates sea pen salmon culture by a number of criteria, including financial feasibility, and concludes that salmon farming can be profitable, contribute to growth, absorb labour, and earn foreign exchange. A final section explores policy options available to governments and to private sector producers.

L'élevage industriel de saumons dans les provinces Maritimes s'avère une source potentielle de développement, d'emploi en milieu rural et d'apport de devises. Le Canada a pris un certain retard sur des pays plus prompts à développer leur aquaculture. Le saumon de l'Atlantique (*Salmo salar*) pourrait être une des espèces donnant au Canada comparativement certains avantages et lui permettant de rattraper son retard. Cet article examine une des techniques d'élevage du saumon, celle des parcs en mer. Il évalue cette technique à partir d'un certain nombre de critères, dont celui de possibilités financières et conclue que l'élevage du saumon peut être profitable, contribuer au développement des régions Maritimes, créer des emplois et permettre une entrée de devises. Une dernière partie explore les options dont disposent les gouvernements et les producteurs du secteur privé.

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Atlantic Canada has the most diverse aquaculture in Canada with six species of fish being commercially produced and another eight in various stages of research. Of these, cage-reared Atlantic salmon (*Salmo salar*) has seen the most dramatic growth, from 6 metric tonnes (t) in 1979 to 150 t in 1982. The approximate value of cage-reared salmon in 1982 was $1.5 million. In the Saint John Fundy Region of New Brunswick alone there are now eight commercial salmon farms, and the area suitable for farming could, at a conservative estimate, produce 10 000–15 000 t a year, some five times more than the total Canadian wild catch of Atlantic salmon (Anderson 1980). Norway and Scotland, with an output of Atlantic salmon of 10 000 and of 3000 t, respectively, give an indication of the potential of commercial farming. By 1985, output is projected to increase by approximately 50% in both countries (University of Stirling 1981).

This paper analyzes fish farming in New Brunswick for its socioeconomic impact. Using microeconomic criteria I indicate the potential employment and foreign exchange earnings that Atlantic salmon farming could induce. Lack of data from fish farms in New Brunswick preclude definitive conclusions, but farming appears to be potentially profitable and a significant earner of foreign exchange. Its potential impact on employment is positive but less significant. These are net benefits, in that any foreign exchange or employment gained are not at the expense of the commercial fishing industry, given the decline in the landings of Atlantic salmon and the possibility of a moratorium my member countries of the North Atlantic Salmon Conservation Association. The paper examines some of the constraints on Atlantic salmon farming, and certain policy issues using Norway and Scotland as illustrations.

The Salmon Market

The output of farmed Atlantic salmon has increased dramatically, both in absolute numbers and share of total Atlantic salmon supply. In 1976, output of farmed salmon was approximately 1600 t with a share of the total supply in western Europe of 18% (the commercial catch accounting for 82%) (University of Stirling 1981). In 1980, output of farmed salmon had more than doubled, and by 1983 had more than doubled again. Output of farmed salmon in 1982 was approximately 10 000 t in Norway and 3000 t in Scotland. The share of farmed salmon in the total supply of Atlantic salmon to western Europe had increased to 44% in 1980 and perhaps to 60% in 1982. That year, 1982, was the first year in which farmed salmon output exceeded wild salmon landings in Britain. As shown in Table 1, Norwegian and Scottish supplies to western Europe are forecast to increase, both in tonnage and as a share of the total. In addition, Icelandic production of farmed salmon is projected to reach 1000 t by 1985, and Ireland's output of 60 t in 1980 should grow sharply with the expansion there of the Norwegian company Mowi.

Demand for farmed Atlantic salmon has been strong. More than 80% of Norwegian farmed salmon is exported, primarily to other western European countries, but also to more distant markets as the United States and Japan. Total export earnings in 1983 could exceed U.S.$100 million (National Marine

TABLE 1. Actual and forecast supplies of fresh and frozen fish to western Europe (compiled from Bulls-Berg 1981; University of Stirling 1981).

	1980 actual		1985 forecast		1990 forecast	
	Tonnes[a]	%	Tonnes[a]	%	Tonnes[a]	%
Farmed						
Scottish	600	1.2	5 000	8	8 000	11
Norwegian	4 300	9.2	15 000	23	18 000	25
Other	—		1 000	2	2 000	3
Total	4 900	10.4	21 000	33	28 000	39
Wild	8 000	17.1	8 000	12	8 000	11
Total Atlantic	12 900	27.5	29 000	45	36 000	50
Pacific imports	34 000	72.5	34 000	55	34 000	50
Total salmon	46 900	100	63 000	100	70 000	100

[a]Live product weight equivalent.

Fisheries Service 1983). From the approximately 700 t exported to the United States in 1982, exports could reach 2000 t in 1983 (worth U.S.$20 million). The Norwegians have succeeded by aggressive marketing. Farmed fish is now more expensive on the market than wild salmon. This is because farm salmon has more fat and is therefore better for smoking; it is also because of butchering techniques and strict quality control. An additional factor has been the winter availability of farmed salmon. The Norwegians have also succeeded in differentiating their product from their competitors'. Atlantic salmon is now ranked ahead of Pacific salmon and Norwegian salmon ahead of that from other countries.

Demand for Atlantic salmon farmed in the Maritimes would come principally from central Canada and the United States. The United States market for fresh salmon remains strong and prices (particularly given the exchange rate) attractive. Even with 2000 t exported to the United States in 1983 the Norwegians would still only account for 2% of the United States domestic market for all salmon. Moreover, our transport costs would be lower than that of the Norwegians. The bulk of Norwegian exports are of fresh (rather than frozen or smoked) fish. The Norwegians are precluded (by 14% tariffs) from exporting smoked fish to the Common Market, and the tariff of 4% on fresh salmon exported to the EEC is not levied, since demand exceeds supply. Since the quality of fresh fish deteriorates rapidly, the Norwegians must depend on air freight, and this transport cost exceeds 10% of the fresh-off-the-boat price of the fish to the New York market and more than 20% to Japan. Atlantic Canada's propinquity to the northern United States should yield it a competitive advantage. One estimate of the potential demand for Atlantic salmon from Canada is 700 t within the domestic market and 3000 t from the United States (Woods-Gordon 1983).

Canada increasingly has concentrated on exporting fresh rather than frozen salmon, with more than 90% of fresh salmon exports going to the United States. The volume of frozen salmon exports has shrunk to a quarter of its 1983 level, and this, combined with a falling (absolute and real) price since 1980, has led to declining revenues (Table 2). Even in current dollars, frozen exported Atlantic salmon in 1983 earned less than a quarter of the 1980 total, barely a third of the 1973 total. The situation for fresh salmon is more positive. As indicated in Table 2, the real price has increased between 1973 and 1983, although with some fluctuations. In addition, the volume and share of fresh salmon exports have increased. Between 1973 and 1983, fresh Atlantic salmon exports almost doubled in volume, more than quadrupled in value, and as a share of total salmon exports rose from 11 to 51%.

Clearly, a critical variable with an expanded supply of fresh Atlantic salmon is elasticity. For if the quantity demanded of salmon were insensitive to price changes, an expanded supply would sharply reduce the price of salmon and thus the income of producing farms. Similarly, if demand were insensitive to income changes, higher real disposable incomes would not increase the demand for salmon, thus threatening producer incomes. The evidence, however, suggests that demand for Atlantic salmon is sensitive to prices and incomes (price and income elastic). This is what the Bay d'Espoir study "strongly suspected" (Marine Sciences Research Laboratory 1980). For fresh and frozen salmon (Atlantic and Pacific), Bell (1978) estimated price elasticity of demand in the United States at -1.3 and income elasticity at 1.6. For an aggregate fresh–frozen model of the United States demand for Pacific salmon, Devoretz (1980) estimated price elasticity of demand at -2.6 and income elasticity at 3.7. As Devoretz noted, one could expect a separate market (e.g. that of fresh Atlantic salmon) to have an even higher elasticity value than that of the aggregate. A recent market study indicated that Atlantic salmon faced high price elasticity of demand (Woods-Gordon 1983).

An Economic Evaluation

There are at least four different techniques for the culture of salmon. Sea cage is based on the premise that salmon being migratory will return to their point of origin (Thorpe 1980). A return of 17.6% of (pink salmon) smolts has been recorded, but Atlantic salmon have shown relatively low return rates; to be economically viable, at least 5% of the smolts released must return as marketable salmon (Stokes 1982). A second technique is that of enclosures, which are natural or man-made, between islands or inlets. The initial investment is high, but operating costs are low, since the enclosures may never have to be renewed. The largest enclosure of Atlantic salmon is that of Mowi's Veløy plant in western Norway with a 200 000 m^3 volume and an annual production of 1000 t. There are four or five Norwegian farms using enclosures. Sea cages are a third method; this is the most common, both in Norway and Scotland. Cages are moored in seawater that is unpolluted and protected from storms. Usually 500 m^3, each cage can hold at harvest approximately 1200 salmon. Sea cages are cheap to install compared with enclosures, but they must be renewed every 5 yr. Western Norway with its sheltered inlets and temperate seawater (between 4 and 18°C) is uniquely suitable for sea changes, while cages in Scotland tend to be more robust, since the area is less sheltered. A fourth technique employs tanks situated on land to which salt water is pumped. Tanks solve the problem of cold water, since the water within can be heated (if necessary); however, they are clearly expensive to purchase and install; electrical costs for pumping are also high.

The technique chosen for the microanalysis in this study considers only sea cages as the production units. As mentioned above, this technique predominates in terms of the number of farms using them, both in Norway and Scotland; it is also the technique used in most of the farms presently operating in New Brunswick. The plant scheduled for Drury Cove (in the Kennebecasis Bay) plans on enclosures, and the Clifton Royal

TABLE 2. Canadian exports of Atlantic salmon 1973–83 (calculated from Statistics Canada (annual), *Exports by commodity and country*, catalogue 65-202).

Year	Volume (t)[a]			Value ($000)			Real price index[b] (1973 = 100)		
	Fresh	Frozen	Total	Fresh	Frozen	Total	Fresh	Frozen	Total
1973	79	621	700	264	2193	2457	100.0	100.0	100.0
1974	125	292	417	420	974	1394	90.7	85.3	85.9
1975	82	467	549	338	1714	2052	100.4	84.6	86.7
1976	57	596	653	295	3554	3849	117.2	128.0	127.2
1977	73	575	648	437	3434	3871	125.6	118.6	119.4
1978	83	363	446	494	2067	2561	114.8	103.9	105.4
1979	103	175	278	870	1347	2217	149.2	128.7	134.1
1980	129	474	603	927	3681	4608	115.2	117.8	116.6
1981	197	401	598	1521	2667	4188	110.0	89.7	95.0
1982	123	268	391	988	1534	2522	103.3	69.7	79.0
1983	152	148	300	1392	761	2153	118.8	59.4	83.5

[a]Quantity refers to whole dressed; a conversion factor of 1.15 should be applied to convert to live product weight.
[b]Index of prices divided by the Consumer Price Index (all items).

farm uses tanks, but those at Deer Island, Grand Manan, and Campobello Island operate with sea cages.

Using 1983 data from Norway and an assumed farm of 3000 m^3, sea cage culture of Atlantic salmon is profitable. At a 15% discount rate the net present value over 10 yr is Can$250 000; the internal rate of return is 31% (Ridler 1983). The high rate of return is typical of Norwegian experience. Annual returns from farms can exceed 100% of the investment (Edwards 1978). Salmon farming is referred to as a "miracle sector" second only to petroleum, and when in 1981 the last licences for salmon farming were issued the 54 available licences attracted 795 applications. It should be noted that a cash flow difficulty exists in the early years of an operation; for 2 yr, operating costs exceed revenues. Also, a 31% rate of return probably overstates profit potential in the Maritimes. Risks in Norway are known and techniques well proven. In addition, the estimate is based on assumed rates of fish growth and survival that may be too high for Atlantic Canada. Yet it provides some indication of the potential profits of salmon farming, and of the need for working capital in the early years.

For economic (rather than financial) evaluation, criteria would include a project's impact on such goals as growth, labour absorption, and foreign exchange earnings. The criteria are given below where Z = total revenues, C is total cost, C_L is the cost of labour, C_D is the cost of domestic capital inputs, C_M is the cost of imported inputs, i is the discount rate, and subscript t refers to year t:

Growth:

$$\sum_{t=1}^{t=10} \frac{Z_t - C_t}{(1+i)}$$

Labour absorption:

$$\sum_{t=1}^{t=10} \frac{C_t}{\text{man-year}}$$

and

$$\sum_{t=1}^{t=10} \frac{C_t}{C_L t}$$

Foreign exchange earnings:

$$\sum_{t=1}^{t=10} Z_t \geqslant \text{or} \leqslant \sum_{t=1}^{t=10} C_M t$$

and

$$\sum_{t=1}^{t=10} \frac{C_L t + C_D t}{(1+i)^t} \Big/ \sum_{t=1}^{t=10} \frac{Z_t - C_M t}{(1+i)^t}.$$

Growth

The criterion for growth is similar to that of the internal rate of return except that shadow (rather than market) prices should be imputed. The most obvious price that needs adjustment is that of labour; and using the Little–Mirrless formula the shadow wage would not merely reflect labour's marginal product of labour but also (assuming a neoclassical saving function) the social cost of consumption rather than saving. If one uses a shadow wage of $3.50 per hour and the cost data above, the effect of the shadow wage is to increase the returns of fish farming, but only marginally. Labour (including an imputed wage to the owner–operator) accounts for 25% of operating costs in the first year and less than 15% thereafter. The return exceeds 35% (rather than 31% of the internal rate of return). This is high and indicates that farming should have a significant impact on the growth and development of Atlantic Canada.

Labour Absorption

One of the attractions of salmon farming to governments concerned about unemployment is its potential for providing jobs. However, salmon farming is not a labour-intensive occupation. Two estimates are made for farming's ability to absorb labour, one using estimated data for a 50-t farm at Deer Island and the other for a 50-t operation in the Kennebecasis Bay (Sutterlin et al. 1981; Anderson Consultants Inc. 1982). Both projects use sea cage techniques. The results are given below:

	Deer Island	Kennebecasis Bay
$\sum_{t=1}^{t=10} \frac{C_t}{\text{man-year}}$	$131 250 (1980)	$74 314 (1982)
$\sum_{t=1}^{t=10} \frac{C_t}{C_L t}$	15%	11%

TABLE 3. Internal exchange rate of 50 t (cage culture) (data from Anderson Consultants Inc. 1982).[a]

Year	Net foreign earnings ($000)	Discount factor 15%	Present worth ($000)	Total domestic costs ($000)	Discount factor 15%	Present worth ($000)
1	−16.1	0.870	−14.0	246.5	0.870	214.5
2	338.4	0.756	255.9	213.1	0.756	161.1
3	340.9	0.658	224.4	203.1	0.658	133.7
4	344.2	0.572	196.9	215.6	0.572	123.4
5	340.9	0.497	169.5	208.1	0.497	103.5
6	337.2	0.432	145.7	226.3	0.432	97.8
7	335.1	0.376	126.0	205.2	0.376	77.2
8	334.2	0.327	112.6	203.1	0.327	66.5
9	340.9	0.284	96.9	203.1	0.284	57.7
10	344.2	0.247	85.1	205.2	0.247	50.7

[a]Internal exchange rate = present worth of total domestic cost/present worth of net foreign earnings = $1086.1/$1399 = $1086.1/U.S. $1136.5 (U.S. to Can. conversion at 1.231).

The first criterion indicates that each man-year of employment costs approximately $100 000, while the second indicates a low capital–labour ratio. The results confirm the experience of Norway. Mowi, with an annual output of more than 500 t of salmon, has created 20 direct jobs; a large farm of 200 t will only generate four or five jobs.

On the positive side it should be noted that the aggregate impact on employment can be significant. In 1982, Scottish salmon farming directly employed 297 people (of which 65 were part-time), and another 200 were employed indirectly in related activities (Scottish Salmon Growers Association 1983). If one assumes a similar output–labour ratio, approximately 900 direct jobs have been created in Norwegian salmon farming and another 600 indirectly, for a total of 1500. In addition, much of the employment is in isolated communities and of people relatively unskilled. Whether in the Highlands of Scotland or northern Norway, salmon farming has been a means of providing employment where few alternatives exist; in Norway, 43% of salmon farmers and their employees came from the fishing sector, and 17% from agriculture (Gerhardsen and Bringsuor 1981).

Net Foreign Exchange Earnings

As explained under *Growth*, salmon farming in the Maritimes would be oriented towards exports, particularly towards the United States. One criterion would estimate whether the value of inputs imported during the 10 yr is less than the value of exports. If the value of inputs imported is less, foreign exchange has been saved. A preferable criterion is the internal exchange rate. It discounts foreign exchange savings and also takes into account the domestic cost of saving foreign exchange. If the cost of converting domestic resources into foreign exchange is less than the official exchange rate, fish farming saves foreign exchange.

In the estimate below, two assumptions are made: first, all fish pen equipment is imported but all remaining expenditures are on domestically produced goods, and second, two-thirds of the output is exported. Sea cages are domestically produced, but the first assumption makes allowance for other imported equipment. It also errs on the side of caution, producing a more conservative estimate. The latter assumption appears plausible; the recent study on market opportunities projected that more than three quarters of salmon output could be exported to the United States by 1990 (Woods-Gordon 1983). However, it may be too high (Anderson Consultants Inc. 1982). Hence, the results will be varied for two rates of exports, that of one third exported and the other of two thirds exported.

Using the first criterion

$$\sum_{t=1}^{t=10} Z_t \geqslant \text{or} \leqslant \sum_{t=1}^{t=10} C_M t$$

and the data of Table 3, a 50-t sea cage farm over 10 yr would make a net foreign exchange contribution in excess of $2 million (1982) if two thirds of the output is exported. With the lower proportion exported (of one third) the net contribution is reduced but still positive, at over $900 000. Using the second criterion, that of the internal exchange rate, the evaluation again was positive; salmon farming is an efficient means of earning foreign exchange. The official exchange rate (left to market forces) converts domestic costs into foreign exchange; if the cost of converting domestic resources into foreign exchange in salmon farming is less than the official rate, the project is efficient at earning foreign exchange. Using a 15% discount rate the internal foreign exchange rate of the sea cage farm is at parity with the American dollar, as shown by Table 3. This is below the official exchange rate; hence, salmon farming is an efficient means of earning foreign exchange. With a shadow wage below the market wage, the relative efficiency of farming is enhanced.

This section on sea cage salmon farming indicates its potential profitability and its potentially significant impact on growth, on foreign exchange earnings, and to a lesser extent on employment. Salmon farming is technically feasible in New Brunswick and has enjoyed rapid expansion, with output more than doubling in each of the last 3 yr. Given the lack of data on existing operations, conclusions must be tentative, but one can speculate that salmon farming could take advantage of Atlantic Canada's resources and skills and generate income, employment, and foreign exchange.

Policies

The recent National Conference on Aquaculture advocated that governments must assist the private sector if aquaculture is to "catch up" with other producing countries. Annual output of cultivated fish is 900 000 t in Japan, 180 000 in the United

States, and a mere 5000 t in Canada (Anderson and Levi-Lloyd 1983). In Europe, freshwater trout production alone now exceeds 110 000 t a year. The argument for government assistance lies in the infant-industry nature of fish farming in Canada and possible socioeconomic externalities. Fish farming differs from the commercial fisheries in not being a common resource. Public funding for perhaps smolt production (and release) may be justified in the commercial fisheries, since there would be no incentive for free enterprise, but not in fish farming. However, public financial assistance for private commercial farms may be justified if, due to their novelty, fish farms are unable to obtain financing from (private) institutions. In addition, government intervention may be justified if there are nonmonetary goals (i.e. the desire to provide employment in isolated communities). In Norway, for example, the priority given to regional development has prompted government financial assistance to, and has determined the number, size, and location of, fish farms. This section briefly examines certain policy problems of Atlantic salmon farming, using Norway and Scotland as illustrations.

Lead Agency

Both the National Aquaculture Conference and the Industry Task Force on Aquaculture have urged that one federal government agency be established as a "lead agency" for aquaculture (Science Council of Canada 1984). By designating such an agency, jurisdictional conflicts could be resolved, a national policy for aquaculture developed, and research stimulated and coordinated. Canada lags behind most other industrialized countries in aquaculture; for it to compete a development program is needed with emphasis on biological and technical research and analysis of financial and economic viability. Such research is not at the expense of the commercial fisheries, since certain species such as Atlantic salmon appear to be doomed as a commercial catch. Farming of Atlantic salmon should be viewed as complementary to the commercial fisheries, as in Norway. There, commercial fishermen have been encouraged to establish fish farms, as their traditional fisheries have become depleted. Research is needed in the areas of site selection, fish growth, economies of scale, and marketability; coordination of such research would be the responsibility of the lead agency.

Financial Assistance

In Norway, governments make few grants for capital investment but offer financial assistance towards operating expenditures at the early stages of operation. For the first 2 yr of the Norwegian fish farm costed in this paper, net revenues were negative; for 5 yr the farm did not offset these negative flows, even when undiscounted. When discounted at 20% the farm's early losses were only recouped in the seventh year. Thus, farms face a severe cash flow problem, unless assisted. Even for the largest farms (e.g. Mowi), survival was ensured only by government guarantee for bank loans. In New Brunswick, venture capital and bank credit for fish farming is still difficult to obtain; hence, governments have been obliged to assist, either by grants or by loans. The need for financial assistance should be reduced as farming becomes established and the risks become known.

Marketing Boards

Quality control in the butchering and marketing of salmon explains in large part the success of the Norwegians. Not only have they succeeded in marketing Atlantic salmon as preferable to Pacific salmon and farmed salmon as superior to wild salmon (with a corresponding price differential), but they market their salmon as a better quality product compared with Scottish (or Canadian) salmon. The marketing board oversees the quality control both for domestically sold fish and those destined for export. Standards of bleeding, handling, and packaging are high, as are requirements for colour and texture. Quality control is also stressed by the Scottish Salmon Growers Association. A further role of the marketing board in Norway is the establishment of a minimum price. All producers are obliged to sell to the marketing boards, which buys the fish at an agreed price; those (e.g. Mowi) who wish to export must repurchase their fish. The marketing board's pricing policy has ensured stability in the salmon market and prevented sharp fluctuations in prices and farm incomes.

Socioeconomic Externalities: Political Issues

Two unique aspects of Norwegian salmon farming are the government's priorities towards regional development and the establishment of licenses. No farm may begin without a license, and the number (and size) is strictly limited. The last issue of licenses was in 1981–82; only 54 were issued, and the maximum size of new farms was 3000 m^3 (approximately 60 t a year). The result of the limitations has been to encourage production by small farms and throughout the northwest of Norway. The criteria for selection are financial viability, self-ownership and operation, and regional location. There are now more than 400 licensed farms in Norway, with the majority less than 5000 m^3 and with more than a quarter located in the three northern counties, Finnmark, Troms, and Nordland. The majority of fish farmers in the northern counties have no other major employment (Gerhardsen and Bringsuor 1981). Fish farming therefore has been a means of retaining population in isolated communities; thus, areas such as Finnmark and Troms are favoured in the issuing of licences. With the new Conservative government, the number and maximum size of new farms (5000 m^3 in 1983 and 800 m^3 in 1984) are expected to increase. Fear of low price elasticity and oversupply had prompted the size limitations; now, recognition of economies of scale (at least up to 200 t) and of the market potential has encouraged the easing of controls.

Scotland has followed a *laissez-faire* approach to salmon farming. As a result, farming is concentrated among a few producers, often subsidiaries of multinational companies (e.g. B. P. and Unilever). Twelve farms account for approximately two thirds of the output. Benefits of this approach are that research and development will be undertaken and the risks borne by private companies rather than governments. Defects are that farming will not include externalities, such as the regional dimension, or the diffusion of research results.

Whether fish farming should primarily be a small-scale operation, with its aim to provide employment to ex-fishermen or farmers, is primarily a political decision. Norway and Scotland have opposite policies. At this time, Norwegian salmon farming is more dynamic than that of Scotland, but this cannot be attributed solely to the approach adopted; Norway began first and also has natural advantages.

Conclusion

This paper has provided a framework for an economic

evaluation of salmon farming and has attempted to illustrate policy options open to governments. Salmon farming is technically feasible in the Maritimes and should be financially profitable. Growing interest in salmon farming suggests that the industry will continue to expand. It provides employment and earns foreign exchange; it also retains population in isolated communities. Policy makers must decide soon if salmon farming is to be a tool of regional policy; otherwise it will develop on the Scottish model with large farms, often foreign owned. Such a model is not necessarily contrary to Atlantic Canada's development but it could involve costs that should be recognized.

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Changes in the Benthic Community of a Small Precambrian Lake following the Introduction of Yellow Perch, *Perca flavescens*

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The introduction and proliferation of yellow perch, *Perca flavescens*, in Little Minnow Lake, Algonquin Park, Ontario, resulted in changes in biomass and size structure of the benthic community. Biomass, mean organism weight, and density data are presented for nine groups of benthic macroinvertebrates for two years before and one year after perch introduction. An approximately 60% reduction in total benthic biomass and an approximately 50% reduction in mean weights occurred in the littoral zone after perch introduction. Vertically migrating profundal benthos showed an approximately 30–60% reduction in mean organism weight but no significant change in biomass. We conclude that fish predation can significantly alter species composition, skew size structure towards smaller invertebrates, and reduce total benthic biomass.

L'introduction et la prolifération de la perchaude, *Perca flavescens*, dans le lac Little Minnow situé dans le parc Algonquin, en Ontario, a entraîné des changements dans la biomasse et la structure de tailles de la communauté benthique. On présente des données sur la biomasse, le poids moyen et la densité moyenne des organismes pour neuf groupes de macroinvertébrés benthiques. Ces données ont été obtenues deux ans avant l'introduction de la perchaude et un an après. On constate, après l'introduction de la perchaude, une réduction d'environ 60 % de la biomasse benthique totale et une réduction d'environ 50 % des poids moyens dans la zone littorale. Dans le cas du benthos de la zone profonde à migration verticale, on observe une réduction d'environ 30–60 % dans le poids moyen des organismes, mais la biomasse ne présente aucun changement notable. En conclusion, la prédation par les poissons peut modifier considérablement la composition des espèces, déplacer la structure de tailles vers des invertébrés plus petits et réduire la biomasse benthique totale.

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Several recent studies on freshwater benthos have led to a controversy as to whether fish predation can significantly alter the benthic community. Thorp and Bergey (1981a, 1981b) showed that the density of benthic organisms and chironomid head capsule lengths were not significantly different between vertebrate predator exclusion cages and control plots. They hypothesized that "individual species of keystone predators do not occur in the littoral zone of freshwater lentic environments with soft bottoms." Similar results were reported by Allan (1982). He found no difference in the density of benthic invertebrates between a section of stream from which 75–90% of the trout (primarily *Salvelinus fontinalis*) had been removed and corresponding upstream and downstream sections. In contrast, Hall et al. (1970) showed that the size selective predation by bluegill, *Lepomis machrochirus*, in experimental ponds significantly decreased the abundance of large species allowing a compensatory increase in smaller species. Crowder and Cooper (1982) showed reductions in benthic biomass and mean organism weight in ponds stocked with bluegill. They suggested that biomass and prey size are "more appropriate response variables" than density for testing the hypothesis that fish predation can significantly alter benthic communities. Crowder and Cooper (1982) also suggested that Thorp and Bergey's acceptance of their general null hypothesis may be inappropriate for two reasons. Firstly, Thorp and Bergey (1981a, 1981b) considered only numerical abundance for all invertebrate groups, with the exception of chironomids for which they also measured size. Secondly, the density of predators excluded from the experimental plots was not presented. Therefore, to address the question of the importance of a predator on the structure of its prey community, quantification of the predation pressure and assessment of factors that reflect the prey community are required. Numerical abundance on its own may not be sensitive enough to reflect the dynamic nature of a community responding to predation pressure.

The present study began in 1967 when yellow perch, *Perca flavescens*, were inadvertently stocked in Little Minnow Lake, a small softwater lake, which had previously supported only hatchery-reared salmonids. After establishment, yellow perch biomass fluctuated yearly between 20 and 30 kg/ha (Fraser 1978). Competition with perch reduced yearly salmonid yield from an average of 8 to 4 kg/ha. The salmonid yield can be

considered as approximately equivalent to biomass, because of the salmonids that survived stocking, most were harvested by intensive angling and gillnetting within 1–2 yr of stocking. Therefore, the total fish biomass of Little Minnow Lake fluctuated yearly between 24 and 34 kg/ha compared with a pre-perch introduction biomass of approximately 8 kg/ha. The purpose of our study was to test the hypothesis that the increase in predation pressure altered the structure of the benthic community by analyzing biomass, size, and density.

Materials and Methods

Little Minnow Lake is located in Algonquin Park, Ontario (45°39′N, 78°21′W). It has an area of 8.9 ha and a maximum depth of 14.4 m. It undergoes summer stratification and loss of hypolimnetic oxygen below approximately 6.0 m. Temperature and oxygen profiles, total dissolved solids, and mean summer surface pH remained constant throughout our study, 1966–80 (J. M. Fraser, Ontario Ministry of Natural Resources, unpubl. data). The salmonid and yellow perch population abundance and feeding ecology have remained unchanged between the post-perch introduction years as described by Fraser (1978) and the fourth year of this study, 1980 (J. M. Fraser, unpubl. data).

The benthos of Little Minnow Lake was sampled eight to nine times from May until August in each of three years: 1966, 1967, and 1979. The benthos was also sampled once in July 1980. A total of 24 samples were taken on each sample data. An Eckman grab was used in a stratified random design. Stratification was by depth, in proportion to the lake surface area covering each of four depth zones: surface–2.9 m, 3.0–5.9 m, 6.0–8.9 m, and 9.0–14.4 m, referred to hereafter as zones 1, 2, 3, and 4. Sample sites were selected randomly within each zone.

Samples were washed in a 500-μm sieve and organisms were hand-sorted live from the remaining material within 24 h of collection. Organisms were identified to class for Annelida and Mollusca, to family for Diptera, and to order for all other taxa using Pennak (1978) and Merritt and Cummins (1978). The number of organisms within each taxon was recorded for each sample. Wet weights were determined by blotting all the organisms within a particular taxon and weighing immediately to within 0.1 μg on a torsion balance. In 1966 and 1967, wet weights were determined by pooling all the samples of a single taxa for each depth zone and for each sample date. Mean organism weight was calculated by dividing weight by numbers for each taxon.

We analyzed the data using the general linear model procedure in the Statistical Analysis System (Freund and Littell 1981). The hypothesis that the benthic communities of 1966, 1967, and 1979 were not different was tested for seasonal means of biomass, mean weight, and density of nine taxa and for the total benthic community for each of four depth zones. The means were calculated from the data collected on each of the eight to nine sample dates for each of the three years. A square root transformation was used to normalize all data (Downing 1979). In cases where the null hypothesis was rejected in the ANOVA ($\alpha = 0.05$), a Student–Newman–Keuls test (Freund and Littell 1981) was used to identify which year or years were different ($\alpha = 0.05$). To address the possibility that the changes noted in the 1979 benthic community were due to a random occurrence rather than to predator effects, the 1980 midsummer data (July 31, 1980) were compared with the data from July 28, 1966, August 3, 1967, and July 30, 1979. These midsummer contrasts could not be tested statistically because variance was not reported for the biomass data of 1966 and 1967; these data were available only as means within each depth zone. Mean weight was calculated by dividing biomass by abundance.

Results

There were significant differences in the Little Minnow Lake benthic community of 1979 when compared with 1966 and 1967; this is illustrated by comparisons of seasonal means of biomass, mean size, and density (Fig. 1). Biomass in zone 1 was approximately 60% lower in 1979. Mean organism weight was reduced by approximately 50% in 1979 in both zones 1 and 4. Density was approximately double in 1979 in zones 3 and 4. To test the hypothesis that these differences in the 1979 benthic community were only by chance, we compared density, biomass, and mean weight of the benthic community for midsummer samples two years before (1977, 1967) and two years after (1979, 1980) yellow perch introduction (Table 1). The midsummer comparisons clearly show a decrease in biomass and mean weight of the 1979 and 1980 communities in zone 1 and an increase in density in 1979 and 1980 in zone 3. Because these comparisons are consistent with those of the

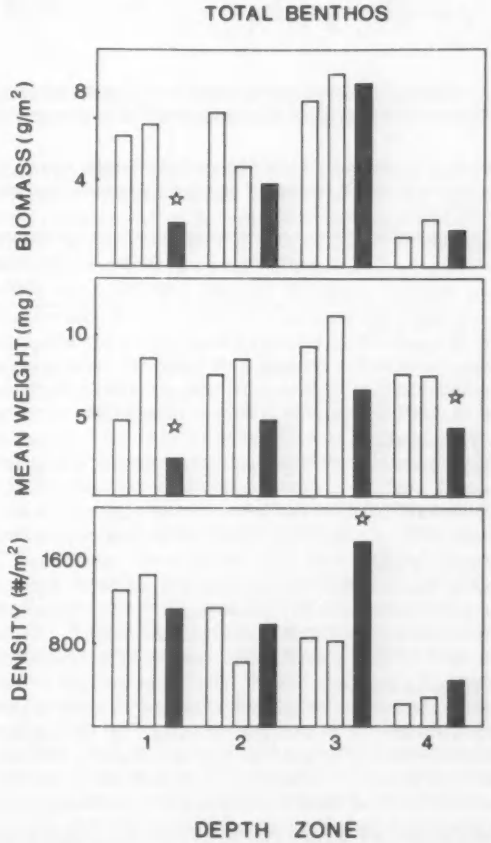

FIG. 1. Biomass, mean weight, and density of the benthic invertebrate community in Little Minnow Lake in 1966, 1967 (open bars), and 1979 (hatched bars) in each of four depth zones. Stars indicate a significant difference ($p < 0.05$) between 1979 and both of 1966 and 1967.

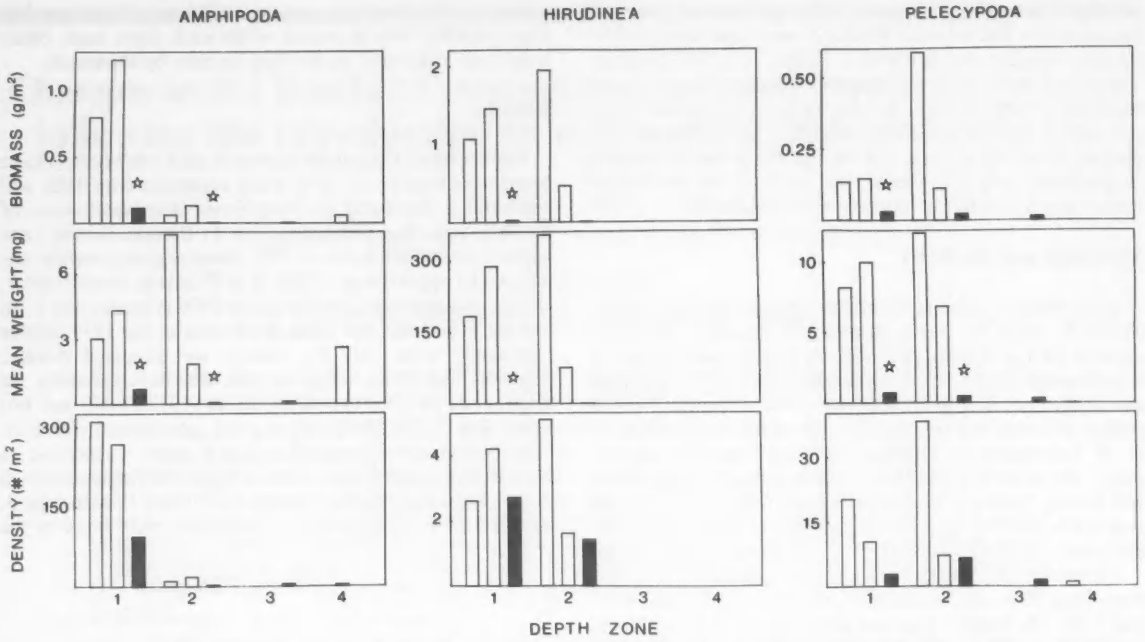

FIG. 2. Biomass, mean weight, and density of Amphipoda, Hirudinia, and Pelecypoda in Little Minnow Lake in 1966, 1967 (open bars), and 1979 (hatched bars) in each of four depth zones. Stars indicate a significant difference ($p < 0.05$) between 1979 and both of 1966 and 1967.

TABLE 1. Comparison of Little Minnow Lake benthic density, biomass, and mean weight for each of four depth zones (standard errors in parentheses).

	Density (no./m²)	Biomass (g/m²)	Mean weight (mg)
Zone 1 (0.0–2.9 m)			
July 28, 1966	1292 (477)	4.02	3.11
August 3, 1967	469 (118)	4.93	10.51
July 30, 1979	2804 (2332)	1.71 (0.08)	0.61
July 31, 1980	1658 (210)	1.86 (0.37)	1.12
Zone 2 (3.0–5.9 m)			
July 28, 1966	720 (300)	4.15	5.76
August 3, 1967	1181 (368)	6.06	5.13
July 30, 1979	780 (134)	3.88 (1.23)	4.97
July 31, 1980	1577 (215)	7.51 (1.54)	4.76
Zone 3 (6.0–8.9 m)			
July 28, 1966	205 (57)	2.52	12.29
August 3, 1967	635 (313)	9.37	14.76
July 30, 1979	1259 (300)	9.88 (2.46)	7.85
July 31, 1980	1227 (139)	4.23 (1.03)	3.45
Zone 4 (9.0–14.4 m)			
July 28, 1966	248 (162)	1.32	5.32
August 3, 1967	140 (77)	1.12	8.00
July 30, 1979	172 (81)	0.98 (0.56)	5.70
July 31, 1980	118 (37)	0.56 (0.19)	4.75

seasonal means, we suggest that the 1979 benthic community is not different by chance but rather that it is a consistent result of the change in the fish community.

The littoral zone groups, Amphipoda, Hirudinia, and Pelycopoda, were all significantly reduced in mean weight and biomass after perch introduction (Fig. 2). None of these groups showed a significant decline in density, although trends are evident in zone 1 for amphipods and pelycopods. Fraser (1978) showed that both amphipods and leeches made up a significant component of the diet of yellow perch in Little Minnow Lake (Table 2). Pelycopods were found in stomachs in trace amounts. We suggest that the abundance of pelycopods was reduced by predation to the point where they were infrequently available to predators. The species composition of the Hirundinea group changed after yellow perch introduction. In 1966 and 1967 the leeches captured were the free-swimming parasitic species whereas in 1979 only scavanger species were collected.

The larval insects, Odonata, Trichoptera, and Ephemeroptera, were all significantly reduced in mean weight in zone 1 in 1979 (Fig. 3). Trends in biomass reduction were nonsignificant. Trichoptera density increased after perch introduction, suggesting a compensatory increase in the smaller species. Density of odonates and ephemeropterans was not significantly reduced. These insect groups were a significant component of the diets of the fish community (Table 2).

TABLE 2. Percentage composition by volume of stomach contents of brook trout, splake, and yellow perch in Little Minnow Lake during spring and summer, 1970–75 (adapted from Fraser 1978).

	Brook trout	Splake	Yellow perch
Amphipoda	0	0	0–20
Odonata	3–15	2–8	2–30
Trichoptera	10–80	2–70	2–15
Ephemeroptera	0–20	0	0–85
Hirudinea	0–10	0	0–5
Pelecypoda	0	0	Trace
Diptera	0–20	5–70	2–10

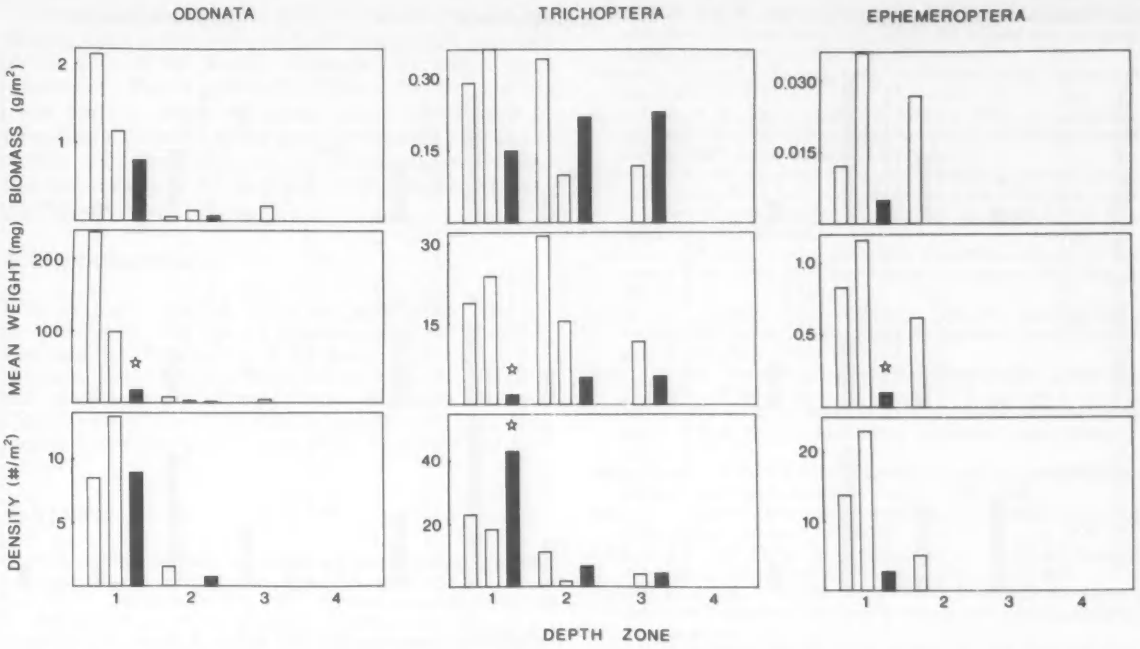

FIG. 3. Biomass, mean weight, and density of Odonata, Trichoptera, and Ephemeroptera in Little Minnow Lake in 1966, 1967 (open bars), and 1979 (hatched bars) in each of four depth zones. Stars indicate a significant difference ($p < 0.05$) between 1979 and both of 1966 and 1967.

The benthic diptera all showed changes in 1979 when compared with the pre-perch years (Fig. 4). The biomass of Chironomidae in zone 1 was significantly reduced as was mean weight in zone 4. Density increased in zones 3 and 4. Chaoboridae mean weight was reduced significantly in zones 2, 3, and 4 with no change in density. Ceratopogonidae biomass and mean weight were reduced significantly in zone 1 and mean weight in zone 2, but there was no density change. Diptera were a significant component of the diet of the fish community (Table 2).

In summary, in the epilimnetic zones after yellow perch introduction, five of nine taxa decreased significantly in biomass, eight decreased significantly in mean weight, and one increased significantly in density (Table 3). In the profundal zones, there were no significant changes in biomass, two taxa were significantly decreased in mean weight, and two taxa increased significantly in density (Table 3).

Discussion

We must reject Thorp and Bergey's (1981a) null hypothesis that fish predators cannot alter their prey community; there were significant differences in the biomass and size structure of the benthic community of Little Minnow Lake between years of low and high predator biomass. Results similar to ours were reported by Crowder and Cooper (1982) for experimental ponds using *Lepomis macrochirus* as the predator. Within 3 wk of fish stocking in a pond with low macrophyte density, benthic biomass was 30% lower and mean individual weights were reduced by 50%. After 3 mo, biomass was reduced by 70% and mean individual weight by 85%. Crowder and Cooper's (1982) low macrophyte pond most closely resembles the epilimnetic zone of Little Minnow Lake, which was described as having "sparse vegetation" (Sprules 1967). In a whole lake study, Ball and Hayne (1952) described a doubling of the total volume of benthic organisms after the removal of the fish community by rotenone poisoning. Hall et al. (1970) found no decrease in benthic biomass in ponds with *L. macrochirus* compared with fish-free ponds, but did describe significant changes among particular taxa. Some species of Chironomidae, Odonata, and Amphipoda were significantly reduced in biomass and mean size in ponds containing fish predators. Mittelbach (1981) attributed the decline in benthic biomass from May until August to selective predation by bluegill on the larger individuals in the community. This reduced availability of preferred prey was reflected in a reduction of stomach content volume corresponding to the reduction in benthic biomass.

Had density been the only variable considered, we would also have mistakenly accepted Thorp and Bergey's (1981a) general null hypothesis. None of the nine taxa sampled showed a significant reduction in density after yellow perch introduction even though there were significant reductions in biomass and mean size. Yellow perch have been shown to feed selectively on large-bodied prey (Keast 1977; Fraser 1978). This size-selective feeding apparently skewed prey size distributions towards smaller bodied individuals, which apparently allowed a compensatory increase in the numbers of small-bodied organisms. This produced a benthic community that was equally dense but lower in total biomass and dominated by smaller bodied organisms. In Little Minnow Lake there were increases in density of epilimnetic trichopterans and profundal chironomids and ceratopogonids after yellow perch introduction. An increase in density of small-bodied groups after predator introduction was also reported by Crowder and Cooper (1982). They attributed the increase in density to the reduction of invertebrate predators by benthivorous fish.

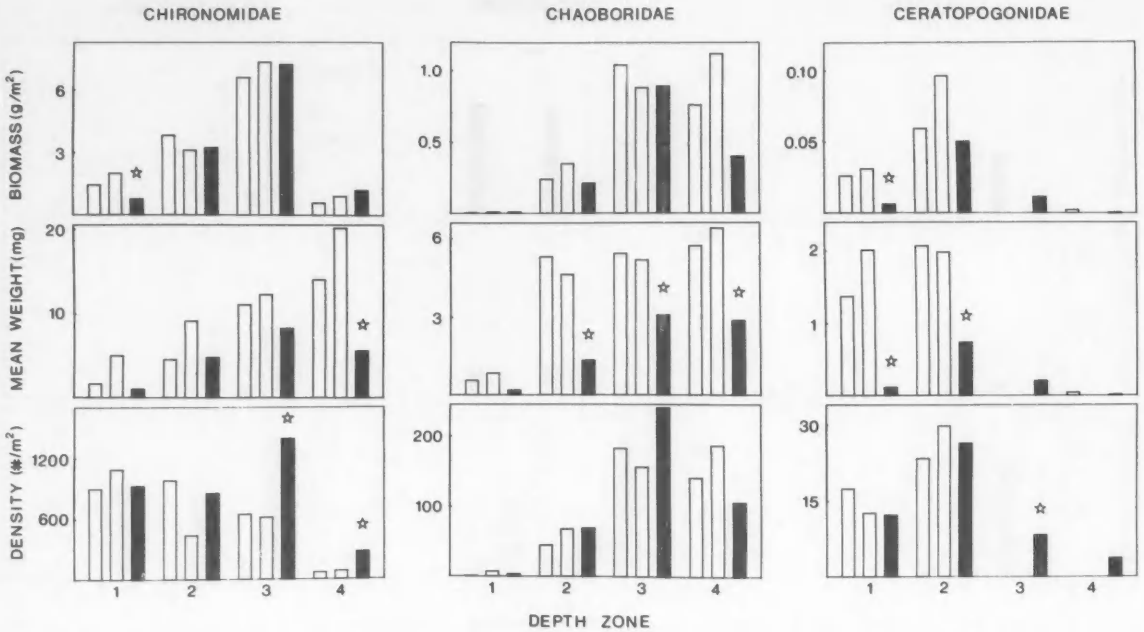

FIG. 4. Biomass, mean weight, and density of Chironomidae, Chaoboridae, and Ceratopogonidae in Little Minnow Lake in 1966, 1967 (open bars), and 1979 (hatched bars) in each of four depth zones. Stars indicate a significant difference ($p < 0.05$) between 1979 and both of 1966 and 1967.

TABLE 3. Significant changes ($p < 0.05$) in biomass, mean weight, and density of benthic invertebrates in the epilimnetic and profundal zones of Little Minnow Lake after the introduction of yellow perch. +, increase; −, decrease; 0, no change; a, absent.

	Epilimnetic zones			Profundal zones		
	Biomass	Mean weight	Density	Biomass	Mean weight	Density
Amphipoda	−	−	0	a	a	a
Odonata	0	−	0	a	a	a
Trichoptera	0	−	+	0	0	0
Ephemeroptera	0	−	0	a	a	a
Hirudinea	−	−	0	a	a	a
Pelecypoda	−	−	0	a	a	a
Chironomidae	−	0	0	0	−	+
Chaoboridae	0	−	0	0	−	0
Ceratopogonidae	−	−	0	0	0	+
Total community	−	−	0	0	−	+

The effects of yellow perch predation on benthic community structure should be most pronounced in the littoral zone where feeding activity of yellow perch on benthos is highest (Keast et al. 1978; Werner et al. 1977). Accordingly, in Little Minnow Lake, biomass and mean weight of the benthos were significantly reduced in the littoral zone in years when yellow perch were abundant. Changes in the benthic community also occurred in the anoxic profundal zones, but this community was dominated by chironomids and chaoborids, both of which are available to feeding fish during noctural vertical migrations (Federenko and Swift 1972; Davies 1976).

Additional evidence supporting our hypothesis that the benthic community of Little Minnow Lake was altered after the introduction of yellow perch comes from the fish community analysis (Fraser 1978). After yellow perch introduction, diets of brook trout shifted from the large benthic invertebrates such as Odonata and Hirudinea to mainly Trichoptera and zooplankton. Splake diets also shifted from fish and Hirudinea to Trichoptera and zooplankton. We suggest that these diet shifts resulted from a reduced availability of prefered prey. Johnson (1977) described the converse situation where white suckers, *Catostomus commersoni*, were removed from a lake where they competed with yellow perch. After sucker removal, adult yellow perch increased their consumption of Ephemeroptera and decreased zooplankton consumption. Their growth rates were markedly increased.

The reduction in abundance and size of benthic prey in Little Minnow Lake is also reflected in the significantly decreased growth rates of the planted salmonids after yellow perch introduction. This is predicted by Kerr's (1977) model and results from a reduced net energy gained from feeding on suboptimal sized prey. Yellow perch growth rates were high in the first years after introduction but then were greatly reduced once they became abundant. Fraser (1978) described the perch population as stunted in growth.

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Tri- and Tetra-Chloroveratrole, Metabolites Produced by Bacterial *O*-Methylation of Tri- and Tetra-Chloroguaiacol: An Assessment of Their Bioconcentration Potential and Their Effects on Fish Reproduction

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Neilson, A. H., A.-S. Allard, S. Reiland, M. Remberger, A. Tärnholm, T. Viktor, and L. Landner. 1984. Tri- and tetra-chloroveratrole, metabolites produced by bacterial *O*-methylation of tri- and tetra-chloroguaiacol: an assessment of their bioconcentration potential and their effects on fish reproduction. Can. J. Fish. Aquat. Sci. 41: 1502–1512.

We have demonstrated that strains of bacteria belonging to the genera *Pseudomonas* and *Acinetobacter* possessed the ability to transform, by *O*-methylation, components of spent bleachery effluent such as 4,5,6-trichloroguaiacol and high molecular weight chlorinated lignin. *O*-Methylation could be enhanced by the presence of a cosubstrate such as succinate or 4-hydroxybenzoate. We have used zebra fish (*Brachydanio rerio*) to examine bioconcentration potential, toxicity to embryos and larvae, and the sensitivity of the offspring of exposed adults. Bioconcentration factors (total wet weight) on a logarithmic scale were 3.5 and 4.4 for 3,4,5-trichloroveratrole and tetrachloroveratrole, respectively. The potential for bioconcentration of the other neutral metabolites was inferred from estimated values of the octanol–water partition coefficients. 3,4,5-Trichloroveratrole, tetrachloroveratrole, pentachloroanisole, and 1,2,3-trichloro-4,5,6-trimethoxybenzene had threshold toxic concentrations to zebra fish embryos and larvae of 450, 100, 2.8, and 450 $\mu g \cdot L^{-1}$, respectively, and all of them were capable of inducing, at somewhat lower concentrations, various deformations including curvature of the larvae and distortion of the notochord. Experiments on the effect of preexposure of adults to tetrachloroveratrole at a concentration of 16 $\mu g \cdot L^{-1}$ showed that the offspring were adversely affected, e.g. in a decreased viability of the embryos, lowered median survival time for larvae, and greater sensitivity to tetrachloroveratrole. Both 3,4,5-trichloroveratrole (5–150 $\mu g \cdot kg$ liver fat^{-1}) and tetrachloroveratrole (40–400 $\mu g \cdot kg$ liver fat^{-1}) were detected in fish from localities subjected to discharge of bleachery effluent, and were absent in fish from uncontaminated localities. Identification of these compounds was based on gas chromatographic, and for tetrachloroveratrole, mass spectrometric, comparison with authentic compounds. We conclude that not only compounds present in bleachery effluent, but those that may be produced in the environment by bacterial *O*-methylation, have potentially hazardous biological activities. These results shed fresh light on a hitherto neglected aspect of environmental hazard assessment, and we suggest that data for a given compound must be supplemented with that for metabolites that could be produced in the environment.

Nous avons démontré que des souches bactériennes appartenant aux genres *Pseudomonas* et *Acinetobacter* possèdent la capacité de transformer, par *O*-méthylation, des composants des eaux usées des blanchisseries de pâte à papier, tels que le 4,5,6-trichloroguaïacol et la lignine chlorée à poids moléculaire élevé. Cette *O*-méthylation peut être accélérée en présence de co-substrats tels que le succinate et le 4-hydroxybenzoate. Nous avons employé des petits danios (*Brachydanio rerio*) pour examiner le potentiel de bioconcentration, la toxicité envers les embryons et les larves et la sensibilité de la progéniture des adultes exposés. Les facteurs de bioconcentration (poids frais total) sur une échelle logarithmique étaient de 3,5 et 4,4 pour le 3,4,5-trichlorovératrole et le tétrachlorovératrole, respectivement. Le potentiel de bioconcentration d'autres métabolites neutres a été inféré d'après les valeurs estimées des coefficients de partage octanol/eau. Le 3,4,5-trichlorovératrole, le tétrachlorovératrole, le pentachloroanisole et le 1,2,3-trichloro-4,5,6-triméthoxybenzene avaient des concentrations seuil de toxicité envers les embryons et larves du petit danio de 450, 100, 2,8 et 450 $\mu g \cdot L^{-1}$, respectivement, et tous avaient la capacité d'induire, à des concentrations encore plus basses, plusieurs types de déformations, par exemple courbure des larves et distorsion du notochorde. Des essais pour déterminer l'effet d'une pré-exposition des adultes au tétrachlorovératrole à une concentration de 16 $\mu g \cdot L^{-1}$ ont montré que la progéniture a été affectée de différentes façons, y compris une baisse de viabilité des embryons, une diminution du temps médian de survie des larves et une sensibilité accrue envers le tétrachlorovératrole. Et 3,4,5-trichlorovératrole (5–150 $\mu g \cdot kg$ matière grasse hépatique^{-1}) et tétrachlorovératrole (40–400 $\mu g \cdot kg$ matière grasse hépatique^{-1}) ont été détectés dans des poissons provenant de localités soumises à des décharges d'effluents de blanchisseries, tandis que ces substances n'ont pas été relevées dans les poissons des localités non contaminées. L'identification de ces composés a été effectuée par comparaison avec des composés authentiques au moyen de chromatographie en phase gazeuse et, pour le tétrachlorovératrole, au moyen de spectrométrie de masse. Nous avons conclu que, non seulement, des composés se trouvent dans les

effluents de blanchisseries, mais aussi ceux qui peuvent être produits dans l'environnement par *O*-méthylation bactérienne, possèdent des activités biologiques potentiellement nuisibles. Ces résultats ont souligné l'importance d'un aspect souvent négligé dans les appréciations du danger des substances chimiques et suggèrent que les données pour une certaine substance doivent être complétées par celles des métabolites pouvant être produites dans l'environnement.

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Conventional bleaching of chemical softwood pulp yields a wide range of chlorinated organic compounds that are formed by a series of complex reactions between chlorine and the lignin contained in the pulp. High and low molecular weight components are included. Chloroguaiacols represent only a fraction of the low molecular weight chlorinated compounds but are discharged in amounts corresponding to 20–30 g·t bleached pulp^{-1} (Lindström and Nordin 1976). Among the quantitatively most significant chlorinated aromatic compounds are 3,4,5- and 4,5,6-trichloroguaiacols, tetrachloroguaicol, and tri- and tetra-chlorocatechols (Lindström and Nordin 1976; Lindström and Österberg 1980; Voss et al. 1980; Holmbom and Lehtinen 1980) and, from mills producing bleached hardwood pulp, also 3,4,5-trichloro-2,6-dimethoxyphenol (Voss et al. 1980; Holmbom and Lehtinen 1980). All of these compounds are, therefore, to be expected in the effluent discharged into receiving waters.

In a previous study, Neilson et al. (1983) showed that pure cultures of bacteria isolated by enrichment from sediment samples metabolized a number of chlorinated phenols and guaiacols with the synthesis of chloroanisoles and chloroveratroles. We have now extended these studies and evaluated the toxicity and bioconcentration potential of these neutral metabolites in laboratory tests using zebra fish (*Brachydanio rerio*). We assessed (1) bioconcentration potential, (2) effect on the mortality of embryos and larvae, (3) induction of deformities in larvae, and (4) adverse effects on the offspring of exposed adults.

We used the results to address an important issue in environmental hazard assessment. Conventional procedures are based exclusively on the properties of the substances under examination and do not take into account the possibility of their transformation in the environment into compounds equally or more toxic than their precursors. Our objective was to evaluate the validity of this assumption.

Materials

The synthesis and assessment of purity of all of the substrates and reference compounds are given in Neilson et al. (1983). However, a more convenient procedure has been used to prepare monomer-free high molecular weight chlorinated lignin from extraction stage (E-stage) liquor. The pH was adjusted to 6.0 and the liquor extracted continuously with diethyl ether during 3 d. Thereby, a stable product, essentially free from chlorophenols and chloroguaiacols, was readily obtained. The pH was then adjusted to 7.0 and the solution sterilized by filtration successively through glass-fibre (Whatman GF/A) and membrane filters (Millipore 1.2, 0.45, and 0.22 μm).

To minimize oxidation of the chlorocatechols, we prepared solutions in deoxygenated water. All of the neutral substances to be examined had only low solubilities in water, and solutions of standardized concentration were prepared as follows. Saturated solutions were prepared by shaking finely divided suspensions at 25°C for 24 h; excess was removed by filtration through glass-fibre filters (Whatman GF/C) and samples were removed for analysis. Appropriate dilutions were then made to provide solutions having uniform and determined concentrations. The approximate solubilities in water (micrograms per litre) at 25°C were as follows: 3,4,5-trichloroveratrole, 2500; tetrachloroveratrole, 700; pentachloroanisole, 60; 1,2,3-trichloro-4,5,6-trimethoxybenzene, 4500. During experiments on embryo–larval toxicity, the concentrations of the test substances diminished during each of the 24-h exposure periods. Analyses were, therefore, made at the beginning and end of each 24-h period, and the mean values were used for calculating the threshold concentrations.

Microbiological Methods

New strains of bacteria were isolated by procedures given by Neilson et al. (1983). Pure cultures were obtained and maintained on slants of nutrient agar (Oxoid) at 4°C or at −70°C in brucella medium base (Oxoid) containing 12.5% (v/v) glycerol. Strains positive for *O*-methylating ability were characterized by our standard procedures (Neilson 1980a, 1980b). Incubation was carried out at 23°C.

Metabolic experiments were carried out with pure cultures. Sampling and substrate additions were made under aseptic conditions, and appropriate controls lacking cell suspensions were included (for details see Neilson et al. 1983). The strains were first screened for their ability to bring about *O*-methylation of 4,5,6-trichloroguaiacol and to produce 3,4,5-trichloroveratrole from the monomer-free high molecular weight chlorinated lignin.

Experiments on the influence of a cosubstrate on *O*-methylation were carried out as follows. Cell suspensions were prepared as already described (Neilson et al. 1983) from cultures grown in media with 4-hydroxybenzoate and succinate as sole sources of carbon. Three sets of incubations were prepared in parallel for each non-growth substrate; in the control culture, the test substrate was incubated with a suspension of cells grown with 4-hydroxybenzoate and without the addition of any other carbon source. For experiments on the effect of the cosubstrates, cell suspensions were prepared from cultures grown with 4-hydroxybenzoate, and a sterile solution of 4-hydroxybenzoate (7.5 g·L^{-1}) added as cosubstrate to give a final concentration of 100 mg·L^{-1}. Succinate-grown cells, with succinate as cosubstrate, were prepared in a similar way. Every second day, cosubstrate was added to provide a nominal concentration of 100 mg·L^{-1}. We estimated that during the 2-d incubation period, the cosubstrate would be completely metabolized. Incubations were carried out with shaking aerobi-

cally in the dark at 23°C. Sampling and analyses for concentrations of the substrates and metabolites were carried out as described by Neilson et al. (1983). Strain 1395 was used for experiments in which 2,4,6-trichlorophenol and 3,4,5-trichloro-2,6-dimethoxyphenol served as nongrowth substrates, whereas strain 1556 was used with 4,5,6-trichloroguaiacol.

Biological Methods

The bioconcentration potential was estimated by three methods. (1) Zebra fish (200–300 mg), obtained from a commercial supplier in Kungsbacka, Sweden, were used in a semistatic procedure in which 80% of the volume of the test solution was replaced daily. Food was supplied twice daily in amounts corresponding to approximately $9\ mg \cdot fish^{-1} \cdot d^{-1}$. The test substances were dissolved in acetone to provide nominal concentrations in the aquaria of approximately 20 μg tetrachloroveratrole$\cdot L^{-1}$ and approximately 10 μg 3,4,5-trichloroveratrole$\cdot L^{-1}$; pure acetone was added to the control aquaria. In experiments with 3,4,5-trichloroveratrole, aquaria of 40 L were used (100 fish) and the exposure time was 4 wk. With tetrachloroveratrole, the exposure time was increased to 8 wk and the volume of the aquaria to 150 L (300 fish). Seven fish were removed periodically for analysis. The bioconcentration factors (BCF's) were calculated from the apparent steady-state concentrations found in the fish, based on the total wet weight and on fat weight, and the mean concentrations found in the water during the whole of the exposure period. (2) Values of the relative mobility (R_M) were measured using C_{18}-HPTLC plates and the octanol–water partition coefficient (P_{ow}) estimated by the method of Renberg et al. (1980a). These were then used to calculate BCF's by the procedure of Mackay (1982). (3) The measured solubilities of the substances in water were transformed into BCF's using the algorithm of Mackay (1982).

The biological assays using zebra fish were based primarily on those of Kihlström et al. (1971) and Speranza et al. (1977); details are given in Landner et al. (1984). Due to possible complications from adsorption of neutral substances to the surface of plastic Petric dishes, tests using eggs and larvae were carried out in glass Petri dishes (100 × 15 mm) containing 50 mL of the test solutions. Experiments were also carried out using the offspring from adults exposed to 16 μg tetrachloroveratrole$\cdot L^{-1}$ during 8 wk before spawning in pure dilution medium. The length of time for which parents are maintained in the dilution medium is of critical significance in determining the outcome of such experiments. Eggs were used that had been released from parents kept for 16–20, 40–44, and 60–64 h in pure dilution medium; these are called regimes I, II, and III.

To determine the threshold toxic concentrations of the substances (i.e. the lowest concentration showing significant toxicity compared with that showing no effect), we analyzed the mortality data using a BioMedical Data Programme *Life tables and survival functions* (Benedetti et al. 1981). This compares the median survival times and their variance for the exposed and the control groups. The *P* values were evaluated by the method of Mantel (1966), which weights more heavily results obtained in the earlier parts of the experiments.

The larvae were fixed in the following buffer at 4°C for histological examination: glutaraldehyde (4%) in cacodylate buffer (0.1 mol$\cdot L^{-1}$, pH 7.3) containing sucrose (3.42%) and cetyl pyridinium chloride (0.5%). They were embedded in glycol methacrylate (JB 4, Polysciences Inc., Warrington) and transverse semithin sections prepared with glass knives in a Reichert ultramicrotome (OM U3). The sections were mounted on glass slides and stained with toluidine blue and alcian PAS.

Analytical Methods

Previously developed methods (Neilson et al. 1983) were used without modification for samples from the microbiological experiments and for the aqueous solutions of the substrates used for the biological tests.

For samples of fish, a more elaborate procedure was required to obtain material sufficiently free from interfering substances. Different procedures were used for zebra fish from experiments on bioconcentration and for fish collected from the Baltic Sea. Four-horned sculpin (*Myoxocephalus quadricornis*) (76 fish: mean weight 114 g), flounder (*Platichthys flesus*) (22 fish: mean weight 325 g), and eel (*Anguilla anguilla*) (16 fish: mean weight 404 g) were collected from three localities subjected to pulp mill discharge containing bleachery effluent. Control fish from three other localities putatively free from such discharge were also examined. In both procedures, the concentration of the internal standard was adjusted to be compatible with that of the substance to be analyzed.

Extraction and Analysis of Fat from Fish

Zebra fish (1.5–3.5 g total wet weight) were homogenized in a mixture of hexane–acetone (1:1, 15 mL), 0.3 mL of phosphoric acid (85%), and 0.5 g of Na_2SO_4. The mixture was allowed to stand overnight and was filtered through a sintered glass filter. The filtrate was saved and the residue extracted with hexane–ether (1:1, 10 mL) containing lindane ($3\ \mu g \cdot L^{-1}$) as internal standard and filtered. The two filtrates were combined and extracted twice with 100 mL of 0.45 mol HCl$\cdot L^{-1}$ containing NaCl (2.0 g). The organic phase was removed and dried (Na_2SO_4). Portions (2.5–5.0 mL) were evaporated to dryness to determine the weight of total fat. This was used for analysis.

Livers (approximately 60 g wet weight) from four-horned sculpin, flounder, and eel and muscle tissue from eel were removed from the appropriate number of fish, cut into small pieces, and extracted (Soxhlet) with acetone during 10 h. Lindane (0.2 μg) was added as internal standard and acetone removed as follows. Hexane–ether (1:1, 100 mL) was added and the combined organic phase washed with 2% aqueous NaCl (2 × 500 mL); the organic phase was dried (Na_2SO_4) and the solvent removed. The weight of the residue (approximately 15 g) was taken as the wet weight of total fat. This was used for analysis.

Fat from zebra fish and four-horned sculpin was hydrolyzed by heating with 1 mL of H_2SO_4 (98%) at 40°C for 2 h. Water (5.0 mL) was added and the mixture extracted with hexane (3.0 mL). The hexane phase was separated and purified by slurrying for 5 min with Sephadex (QAE, A-25, Pharmacia) suspended in 0.1 mol NaOH$\cdot L^{-1}$. The organic phase was removed and used for gas chromatographic (GC) analysis. Fat from flounder and eel was treated as follows. KOH in ethanol (1 mol$\cdot L^{-1}$, approximately 20 mL) was added to the fat and the mixture incubated at 50–70°C for 1 h. Water (500 mL) was added, the mixture extracted twice with hexane–ether (1:1, 50 mL), and the extracts dried (Na_2SO_4) and applied to a column of silica gel (Merck Kieselgel 60, 70–280 mesh). The column was eluted with hexane, the combined eluates concentrated to approximately 15 mL, and the concentrate used for GC analysis.

Identification of the chloroveratroles was based on comparison of the relative GC retention times with those of authentic samples. Mass spectrometric comparison was carried out as previously described (Neilson et al. 1983) except that since the samples were not free from interfering lipids, only a portion of the mass spectrum can be utilized for comparison.

Results

Bacterial *O*-Methylation

Four of the newly isolated bacterial strains were capable of synthesizing 3,4,5-trichloroveratrole both from 4,5,6-trichloroguaiacol and from the high molecular weight chlorinated lignin (Table 1). All four strains were strictly aerobic, Gram-negative, catalase-positive, rod-shaped bacteria. Their biochemical reactions are given in Table 2. The three oxidase-positive strains were assigned to the fluorescent group within the genus *Pseudomonas*, whereas the single oxidase-negative strain was assigned to the genus *Acinetobacter*. A single strain (1559) produced low but significant amounts of tetrachloroveratrole ($110\ \mu g \cdot L^{-1}$) and, in addition, 3,4,6-trichloroveratrole ($20\ \mu g \cdot L^{-1}$) from E-stage liquor.

From the experiments on cometabolism, the final yields of the *O*-methylated products obtained in the presence of different cosubstrates are given in Table 3, and the kinetics of *O*-methylation of 3,4,5-trichloro-2,6-dimethoxyphenol by strain 1395 is shown in Fig. 1.

Analysis of Fish from the Baltic Sea

Tetrachloroveratrole was found in fish from all three localities receiving bleachery effluent and was absent ($<0.1\ \mu g \cdot kg$ total fat^{-1}) in fish from control localities. Concentrations in the liver (micrograms per kilogram total fat) were as follows: four-horned sculpin, 50–400; flounder, 40–70; eel, 50. The structure of the product extracted from fish from one of the localities was confirmed by mass spectrometric comparison with authentic tetrachloroveratrole (Fig. 2). The three parent ion peaks at *m/e* 273.9, 275.9, and 277.9 correspond to those required for tetrachloroveratrole ($C_8H_6O_2Cl_4$). Trichloroveratrole was also found, though in considerably lower concentrations, in four-horned sculpin (5–150), flounder (10), and eel (15). In muscle tissue fat from eel, the concentrations of tetrachloroveratrole and 3,4,5-trichloroveratrole were 45 and $10\ \mu g \cdot kg$ total fat^{-1}, respectively.

Bioconcentration

It has been shown that there is a good correlation between R_M, P_{ow}, and bioconcentration potential (Renberg et al. 1980a; Mackay 1982). Values of R_M and estimated values of P_{ow} are given for a number of neutral metabolites produced by bacterial *O*-methylation of chlorinated phenols and guaiacols (Table 4).

The kinetics of accumulation of 3,4,5-trichloroveratrole and tetrachloroveratrole in zebra fish are given in Fig. 3 and 4. From these, the $\log_{10}$ BCF's were calculated to be 3.5 for trichloroveratrole and 4.4 for tetrachloroveratrole based on total wet body weight or 4.7 and 5.2 based on weight of fat.

Adverse Effects on Zebra Fish Embryos and Larvae

Spontaneous skeletal deformities in adults observed in inbred strains of zebra fish (Piron 1978) have not been observed in our material. Spontaneous abnormalities in larvae were seldom encountered; the frequency of occurrence of curvature in larvae was <0.02% so that unambiguous association of such deformations with exposure to a test substance did not present any difficulty. After direct exposure, values for the threshold toxic concentrations of chlorinated catechols, guaiacols, veratroles, and pentachloroanisole are given in Table 5, together with concentrations inducing curvature of the larvae. A time-dose-response curve is shown in Fig. 5. Representative photographs of deformed larvae, and of semithin sections prepared from these, after exposure to 3,4,5-trichloroveratrole are shown in Fig. 6 and 7. The histological examination consistently revealed deformation of the notochord (Fig. 7B and 7C). Degenerative changes were also observed in the muscle cells of the deformed larvae. Disintegration of the cells occurred with an increase in the extracellular space, and certain areas of the muscle cells had a vacuolated appearance (Fig. 7E). These alterations were dependent on the concentration of 3,4,5-trichloroveratrole, and at higher concentrations, the effects were both more diverse and more severe. Although grossly similar deformations were found

TABLE 1. Final yield ($\mu g \cdot L^{-1}$) of 3,4,5-trichloroveratrole produced by various strains of bacteria after 30 d of incubation of 4,5,6-trichloroguaiacol ($50\ mg \cdot L^{-1}$) and high molecular weight E-stage liquor.

	Substrate	
Strain	4,5,6-Trichloroguaiacol	E-stage
1556	150	30
1557	360	210
1558	200	25
1559	890	250

TABLE 2. Biochemical reactions of Gram-negative bacteria capable of bringing about O-methylation.

	Strain			
	1556	1557	1558	1559
Oxidase (Kovács)	+	+	+	−
Arginine dihydrolase	+	+	+	−
Lysine decarboxylase	−	−	−	−
Ornithine decarboxylase	−	−	−	−
Hydrolysis of:				
ONPG	−	−	−	+
Tween-80	−	−	−	−
DNA	−	−	−	−
Gelatin	−	−	−	−
Gas from nitrate	−	+	−	−
Nitrite from nitrate	−	+	−	−
Acid from (ASS medium)				
Glucose	+	+	+	+
Maltose	+	−	+	+
Xylose	+	+	+	+
Arabinose	+	+	+	+
Mannitol	+	+	+	+
Utilization of				
Glucose	−	+	−	+
Gluconate	+	+	−	+
4-Hydroxybenzoate	+	+	+	+
3-Hydroxybenzoate	−	−	−	−
Cetrimide (0.03%) tolerance	+/−	+/−	+	−
Fluorescent pigment	−	−	+	−

TABLE 3. Concentrations ($mg \cdot L^{-1}$) of *O*-methylated products formed from various substrates ($50\ mg \cdot L^{-1}$) in the presence of succinate and 4-hydroxybenzoate as cosubstrates after 35 d of incubation.

Substrate (strain no.)	Control	Succinate	4-Hydroxybenzoate
2,4,6-Trichlorophenol (1395)	12.5	8.5	16.5
3,4,5-Trichloro-2,6-dimethoxyphenol (1395)	0.3	2.3	1.8
4,5,6-Trichloroguaiacol (1556)	0.3	0.09	0.5

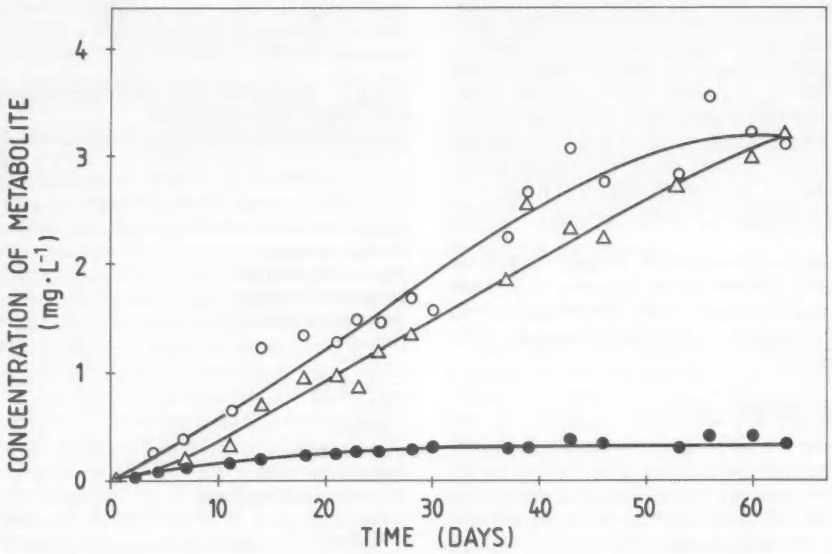

FIG. 1. Kinetics of the synthesis of 1,2,3-trichloro-4,5,6-trimethoxybenzene from 3,4,5-trichloro-2,6-dimethoxyphenol ($50\ mg \cdot L^{-1}$) in the presence of cosubstrates. ●, control without cosubstrate; ○, succinate; △, 4-hydroxybenzoate.

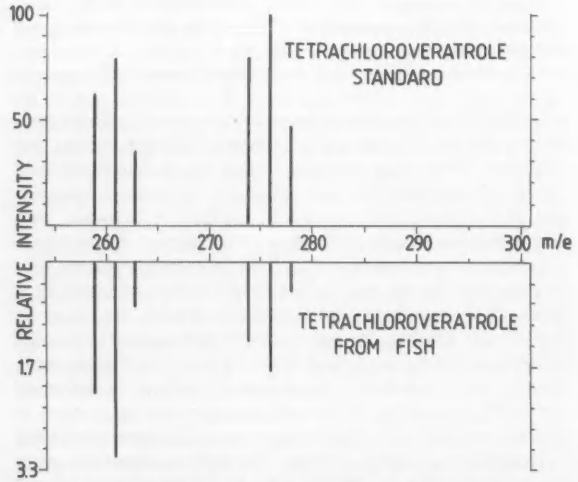

FIG. 2. Partial mass spectrum of authentic tetrachloroveratrole and of the neutral compound isolated from fish liver fat.

after exposure to all of the neutral substances examined, certain differences between them are important, especially after exposure to higher concentrations of the test substances. For example, exposure to concentrations of 500 μg tetrachloroveratrole·L^{-1} induced hemorrhage, clearly shown in Fig. 6E, whereas approximately 20% of larvae deformed after exposure to concentrations of 1800 μg 1,2,3-trichloro-4,5,6-trimethoxybenzene·L^{-1} developed gross dilation of the abdomen (Fig. 6F). Similar dilation was noted also after exposure to 300 μg 3,4,5-trichloro-2,6-dimethoxyphenol·L^{-1}.

The offspring of exposed adults were also adversely affected (Table 6; Fig. 8). When we compared offspring from exposed adults (E group) with those of a control group (C), we observed the following: (1) no significant differences in egg production (regimes I, II, and III), (2) the fraction of nonviable eggs including embryos dying during the first 24 h increased from 12% in the C group to 23% in the E group (regimes II and III), (3) no significant differences in hatching frequencies in pure dilution medium, although embryos in the E group had a significantly lowered median survival time (Table 6, regimes I and II), (4) offspring from the E group had a significantly increased sensitivity to tetrachloroveratrole during the embryo

TABLE 4. Relative mobilities (R_M) and values of log P_{ow} estimated from (1) the R_M values and (2) measured solubilities in water. Log BCF's calculated from the first are also given, and observed values from bioconcentration experiments with zebra fish are given in parentheses. n.d., not determined.

Substance	R_M	log P_{ow} (1)	log P_{ow} (2)	log BCF	
2,4,6-Trichloroanisole	0.59	5.2	n.d.	3.9	
Pentachloroanisole	0.96	6.5	6.9	5.2	
3,4,5-Trichloroveratrole	0.47	4.6	5.25	3.3	(3.5)
Tetrachloroveratrole	0.76	5.8	5.9	4.5	(4.4)
1,2,3-Trichloro-4,5,6-trimethoxybenzene	0.53	4.8	5.0	3.5	

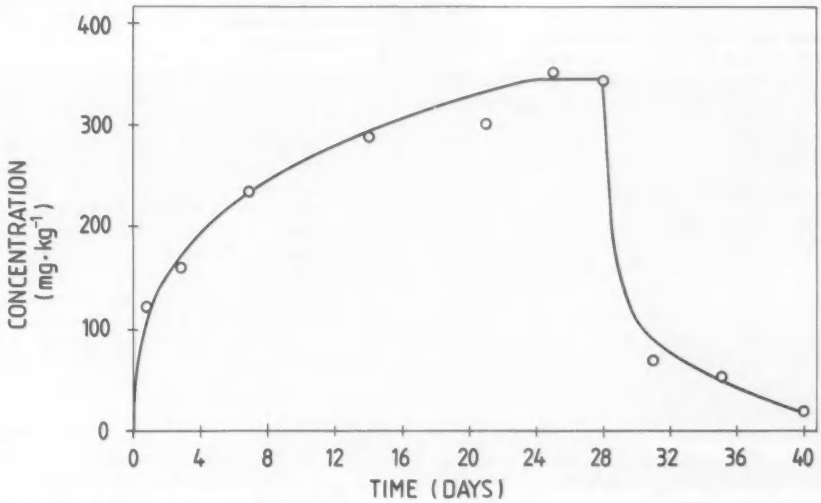

FIG. 3. Kinetics of bioconcentration by zebra fish exposed during 4 wk to a concentration of 7 μg 3,4,5-trichloroveratrole·L^{-1}. Concentrations are given as mg·kg fat $weight^{-1}$.

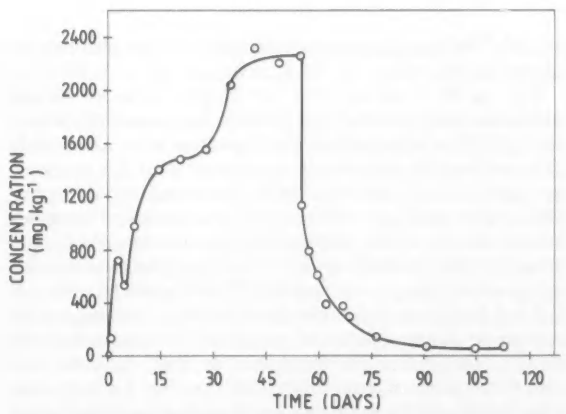

FIG. 4. Kinetics of bioconcentration by zebra fish exposed during 8 wk to a concentration of 16 μg tetrachloroveratrole·L^{-1}. Concentrations are given as mg·kg fat $weight^{-1}$.

and larval stages (Fig. 8, regime III), and (5) the frequency of occurrence of deformed larvae in the E group was twice that in the C group after offspring from both groups were exposed to 30–50 μg tetrachloroveratrole·L^{-1} (regime III).

Discussion

Bacterial *O*-Methylation

Four newly isolated strains of bacteria were able to bring about *O*-methylation; these were Gram-negative rods belonging to the genera *Pseudomonas* and *Acinetobacter*, both of which are widely distributed in the terrestrial and aquatic environments (Palleroni 1981; Juni 1978). Although the yields obtained with the Gram-negative bacteria were lower than those found previously with strains of *Arthrobacter*, they were quantitatively significant.

In an earlier study (Neilson et al. 1983) we showed that 3,4,5-trichloro-2,6-dimethoxyphenol was the principal metabolite from 3,4,5-trichloroguaiacol but not from 4,5,6-

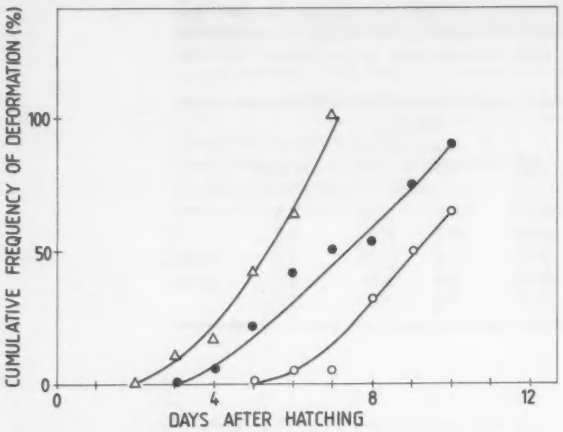

FIG. 5. Time of onset and frequency of occurrence of curvature in larvae of zebra fish exposed to various concentrations of 3,4,5-trichloroveratrole. △, 1200 $\mu g \cdot L^{-1}$; ●, 500 $\mu g \cdot ^{-1}$; ○, 200 $\mu g \cdot L^{-1}$. Unexposed controls had frequencies of curvature of less than 0.02% during the experimental period.

trichloroguaiacol; further *O*-methylation was not observed. We have now shown that the completely *O*-methylated product was produced, though in low yield, and that the yield was enhanced sevenfold in the presence of a cosubstrate. This observation is significant since (a) 3,4,5-trichloroguaiacol is one of the principal chlorinated phenolic components of effluents from mills using pine (*Pinus silvestris*) and spruce (*Picea abies*) and (b) 3,4,5-trichloro-2,6-dimethoxyphenol itself occurs in effluents from mills using birch (*Betula pubescens*).

The extent of *O*-methylation was not always enhanced by the presence of a cosubstrate, and the relative effectiveness of succinate and 4-hydroxybenzoate was variable (Table 3). These observations suggest a role for cosubstrates additional to those envisaged previously (Horvath 1972; Perry 1979).

In summary, our results show that the capacity for *O*-methylation of chlorophenols and chloroguaiacols is not confined to restricted taxonomic groups and also reveal some of the complex interactions that may be encountered in natural situations where a number of substrates are simultaneously available.

Biological Consequences of *O*-Methylation

Previous investigations have demonstrated a number of toxic effects of chlorinated aromatic compounds identified in bleachery effluents (Leach and Thakore 1975; Renberg et al. 1980b; Saarikoski and Viluksela 1982) and the possibility of their bioconcentration (Landner et al. 1977; Renberg et al. 1980b). Our study has clearly revealed an aspect that has not apparently been taken into consideration hitherto. It has generally been assumed that metabolism of a substrate produces compounds that are both more hydrophilic and less toxic than their precursors. Although the metabolites we examined here were clearly not toxic to the bacteria producing them, the situation for other components of the aquatic ecosystem is entirely different. The metabolites have been conclusively shown to possess a number of properties having significant detrimental consequences: (1) they showed a high potential for bioconcentration, (2) they were highly toxic to fish embryos and larvae, (3) they induced potentially lethal deformations in exposed larvae, and (4) they induced an adverse sensitivity in the offspring of exposed adults.

TABLE 5. Threshold concentrations (lowest concentrations showing a significant effect) ($\mu g \cdot L^{-1}$) for embryo/larvae mortality. Concentrations showing no significant effect are given in parentheses. Lowest concentrations ($\mu g \cdot L^{-1}$) inducing curvature of larvae are also given.

Substrate	Toxic concentration	Deformation concentration[a]
4,5-Dichloroguaiacol	500 (300)	—
3,4,5-Trichloroguaiacol	300 (200)	—
4,5,6-Trichloroguaiacol	500 (300)	—
Tetrachloroguaiacol	300 (200)	—
3,4,5-Trichloro-2,6-dimethoxyphenol	800 (600)	—
3,4,5-Trichlorocatechol	250 (200)	—
Tetrachlorocatechol	250 (150)	—
3,4,5-Trichloroveratrole	450 (300)	200
Tetrachloroveratrole	100 (50)	50
1,2,3-Trichloro-4,5,6-trimethoxybenzene	450 (350)	150
Pentachloroanisole	2.8 (2.0)	2.8

[a]—, deformation not observed.

TABLE 6. Survival, in medium lacking tetrachloroveratrole, of offspring from zebra fish previously exposed to tetrachloroveratrole. Egg hatchability (%) and the median survival time (d), together with the standard deviation, of embryos and larvae from exposed and unexposed parents. Eggs released from parents maintained in pure dilution medium for 16–20 h after termination of exposure were used in series A and for 40–44 h in series B. Eight replicates were used in all series of experiments. Wilcoxon–Mann-Whitney test: $^{**}0.01 > P \geq 0.001$; $^{***}P < 0.001$; n.s., not significant ($P > 0.05$, χ^2 test).

Parents	Hatchability	Median survival time	SD
Exposed (A)	97 (n.s.)	13.0***	0.43
Unexposed (A)	99	14.6	0.60
Exposed (B)	100 (n.s.)	13.7**	0.50
Unexposed (B)	100	14.5	0.18

The log BCF values (total wet weight) were 3.5 for the trichloroveratrole and 4.4 for the tetrachloroveratrole. Values for log BCF's estimated from the R_M values were 3.3 and 4.5. These values are comparable with those for 1,2,4-trichlorobenzene (3.3) and *p,p'*-DDT (4.5) (Veith et al. 1979; Mackay 1982; Oliver and Niimi 1983) so that both chlorinated veratroles clearly showed a high potential for concentration in fish, and presumptively, in other aquatic organisms. The same conclusion is drawn from the estimated BCF's for pentachloroanisole and 1,2,3-trichloro-4,5,6-trimethoxybenzene. Although concentrations of the chlorinated veratroles in exposed fish fell rapidly when fish were transferred to fresh medium, low concentrations were remarkably persistent (Fig. 3 and 4). This is consistent with the somewhat low concentrations found in fish collected from the Baltic Sea, and is supported by current observations which have demonstrated demethylation of the veratroles to guaiacols that are excreted into the medium. This

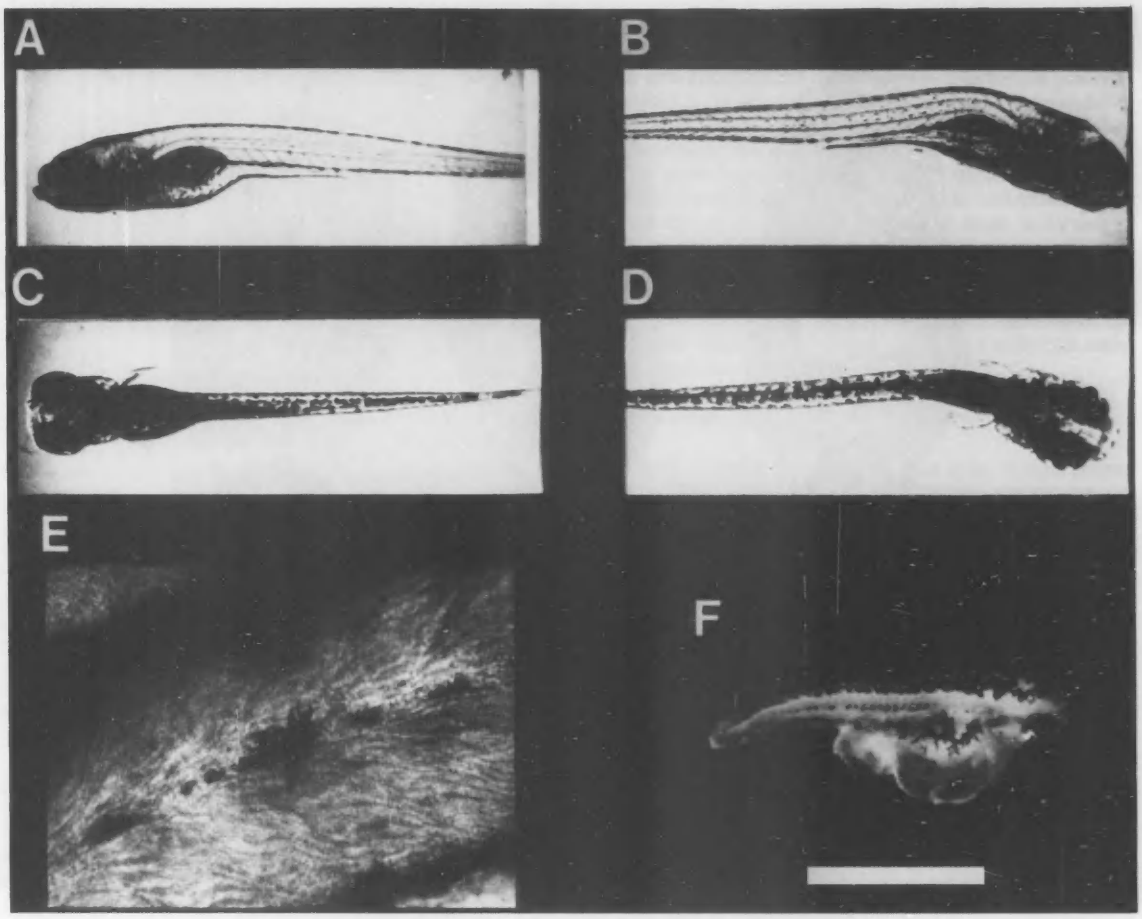

FIG. 6. Zebra fish larvae showing deformities induced after exposure to chloromethoxybenzenes. (A) Control larva (lateral view); (B) curved larva (lateral view) after 7 d of exposure to 1700 μg 3,4,5-trichloroveratrole·L^{-1}; (C) control larva (dorsal view); (D) curved larva, same as Fig. 6B (dorsal view); (E) lateral view of larva deformed after 5 d of exposure to 500 μg tetrachloroveratrole·L^{-1} (hemorrhage in muscle tissue is shown by the dark area at the centre of the photograph); (F) dilation of the abdomen in larva after 5 d of exposure to 1800 μg 1,2,3-trichloro-4,5,6-trimethoxybenzene·L^{-1}. Bar represents 1.0 mm for Fig. 6A, 6B, 6C, 6D, and 6F and 0.1 mm for Fig. 6E.

may be compared with the data of Glickman et al. (1977) for the metabolism of pentachloroanisole in rainbow trout (*Salmo gairdneri*).

Clearly, the toxicity of the veratroles to embryos and larvae is comparable with that of the corresponding guaiacols and catechols (Table 5): the extreme toxicity of tetrachloroveratrole and pentachloroanisole should be particularly noted. Although curvature of larvae was induced after exposure to all of the neutral chlorinated methoxybenzenes (Table 5), this has not been observed after exposure to fresh bleachery effluents, or to any of the chlorinated catechols or guaiacols examined. Degenerative changes in the muscle cells were dose-dependent although muscle tissue was not affected uniformly: it appears that curvature of the body of the larvae and deformation of the notochord are the secondary result of these alterations in the muscle. Extensive damage to restricted areas of the muscle tissue could induce adverse changes in muscle tonus, which would then result in the curvature observed during development of the larvae.

Spinal deformation in adult fish has been found after exposure to substances of such structural diversity as base metal cations (Bengtsson 1974; Holcombe et al. 1976), kepone (Couch et al. 1977; Buckler et al. 1981; Mehrle et al. 1981), and malathion and 2,4-dichlorophenoxyacetic acid butoxyethyl ester (Mount and Stephan 1967). The underlying biochemical mechanisms and physiological consequences may, however, differ. For the organic compounds at least, a number of consequences have been observed, including differences in the mechanical properties of the vertebrae and changes in the amount and composition of collagen isolated from the vertebrae (refs. in Mehrle et al. 1982). We tentatively suggest that the deformities observed in larvae during this study were early stages in the development of analogous spinal deformations in adult fish.

Experiments on the effects of preexposing adults to a concentration of 16 μg tetrachloroveratrole·L^{-1} before testing the offspring revealed a number of disturbing consequences. The threshold toxic concentration determined from these experiments is at least five times lower than that found in

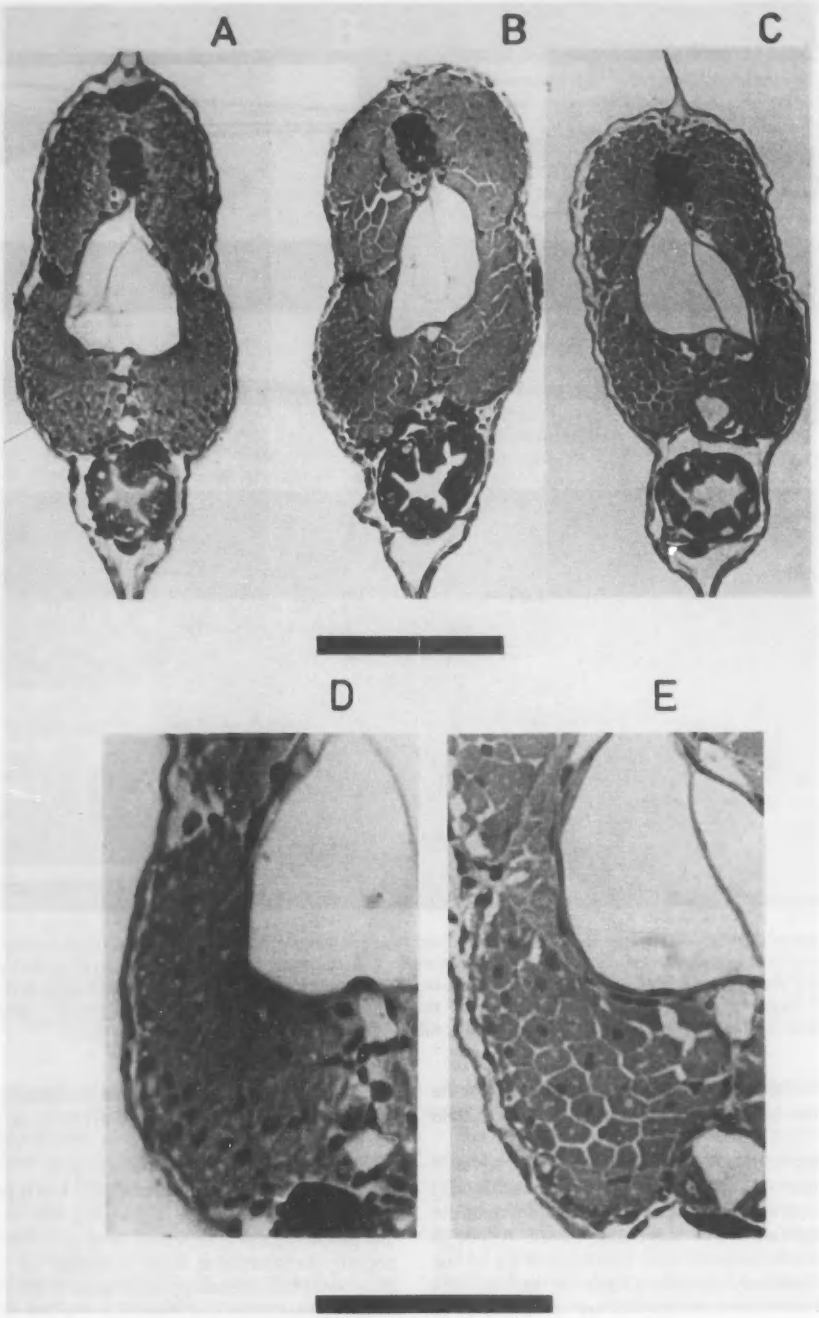

FIG. 7. Semithin transverse sections of zebra fish larvae after exposure to 3,4,5-trichloroveratrole. Sections were made behind the abdominal cavity and were stained with toluidine blue. (A) Control; (B) deformation of the body and notochord after 7 d of exposure to 250 μg 3,4,5-trichloroveratrole·L^{-1}; (C) severe deformation of the body and notochord and derangement of the muscle cells after 7 d of exposure to 750 μg 3,4,5-trichloroveratrole·L^{-1}; (D) control; (E) severe disintegration of the muscle cells, which have a shrunken appearance after 7 d of exposure to 750 μg 3,4,5-trichloroveratrole·L^{-1}. Bar under Fig. 7A, 7B, and 7C represents 0.1 mm and under Fig. 7D and 7E represents 0.05 mm.

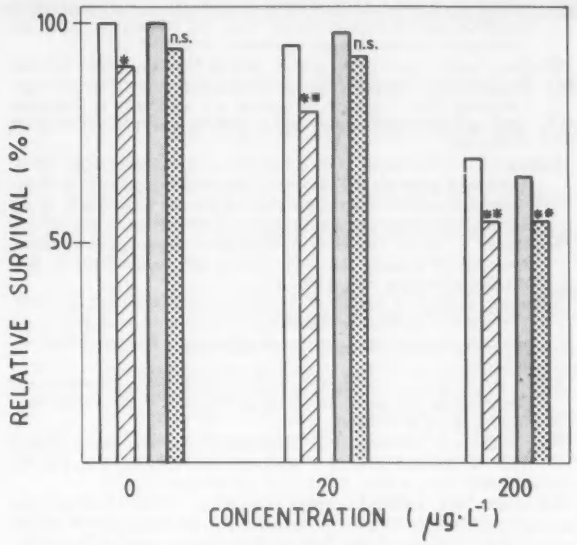

FIG. 8. Comparison of survival times for offspring of zebra fish from parents exposed to various concentrations of tetrachloroveratrole (E) and from unexposed parents (C). Median survival times are given as % of control groups. □, C group, embryo/larvae; ▨, E group, embryo/larvae; ▩, C group, larvae; ▦, E group, larvae. Eggs used in these experiments were from parent fish maintained for 64–68 h in pure dilution medium after termination of the preexposure. $*P < 0.05$; $**P < 0.01$; n.s., not significant.

experiments in which the eggs and larvae were exposed directly. Even extremely low concentrations of the metabolites could then induce effects potentially adverse to the survival of wild fish populations.

Some of the metabolites shown to be toxic in laboratory experiments were also found in wild fish. Analysis of fish from the three localities subjected to discharge of bleachery effluent showed the presence of both 3,4,5-tri- and tetrachloroveratroles although the concentrations were rather low. The identification of the tri- and tetra-chloroveratroles was based on comparison of relative GC retention times with those of authentic samples, and, for tetrachloroveratrole, on the partial mass spectrum (Fig. 2). There remains, therefore, no reasonable doubt about the identity of these substances. These compounds were not detected in fish from uncontaminated localities. These findings are significant because of the unambiguous demonstration of appreciable concentrations of substances having no direct source. Neither substance has been found in raw bleachery effluent, and neither is known to be produced in any industrial operation. To the best of our knowledge, chlorinated veratroles have not been found previously in free-living fish. We conclude that these substances arise from bacterial *O*-methylation of the corresponding chloroguaiacols or high molecular weight chlorinated lignin, all of which are significant components of bleachery effluent.

In a wider perspective, the natural occurrence of microbial *O*-methylation is supported by the results of Renberg et al. (1983), demonstrating the occurrence of pentachloroanisole in the muscle tissue of fish after an accidental spill of pentachlorophenol, and of Watanabe et al. (1983a, 1983b) who showed the presence in mussels and sediment of the di-*O*-methyl ether of tetrabromobisphenol-A. In neither case is an alternative source of the *O*-methylated compounds known.

Our data underline the significance of a factor not generally considered in environmental hazard assessments: the possibility that natural microbial processes may produce metabolites equally or more toxic than the substances discharged originally. Data for a given compound must then be supplemented by data for metabolites that may be produced in the environment.

A number of so far unresolved issues must, however, be examined before a valid extrapolation of the results of laboratory experiments to the natural environment is made. Among these, and clearly of cardinal importance, are the dynamics of the synthesis and distribution of *O*-methylated metabolites and their further transformation in fish, water, and sediments under natural conditions.

Acknowledgments

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Intakes as Sampling Locations for Investigating Long-Term Trends in Zooplankton Populations[1]

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Evans, M. S., and L. F. Flath. 1984. Intakes as sampling locations for investigating long-term trends in zooplankton populations. Can. J. Fish. Aquat. Sci. 41: 1513–1518.

We present data demonstrating the representativeness of a power plant intake as a sampling location for investigating long-term trends in zooplankton populations. The intake (Donald C. Cook Nuclear Plant) and the inshore region (southeastern Lake Michigan) were sampled within a matter of days on 54 occasions between April 1975 and November 1981. Intake population estimates were significantly correlated with inshore region population estimates for all 26 zooplankton taxa categories analyzed. Differences in abundances in intake and inshore region samples were not significant for most euplanktonic taxa. The plant apparently entrained water and microcrustaceans from the sediment–water interface. However, because vertically hauled plankton nets do not effectively sample the lower 1 m of the water column, intake sampling may provide a more accurate representation of nearshore region copepod and cladoceran community structure than traditionally employed methods for inshore region sampling.

Nous présentons des données démontrant la représentativité de la prise d'eau d'une centrale électrique comme point d'échantillonnage pour l'étude des tendances à long terme des populations zooplanctoniques. La prise d'eau (centrale nucléaire Donald C. Cook) et la zone riveraine (sud-est du lac Michigan) ont été échantillonnées, en quelques jours, à 54 reprises d'avril 1975 à novembre 1981. Les estimations des populations de la prise d'eau présentaient des corrélations significatives avec celles de la zone riveraine, ceci pour les 26 catégories de taxons planctoniques étudiées. Les différences d'abondance entre les échantillons de la plupart des taxons euplanctoniques de la prise d'eau et de la zone riveraine n'étaient pas significatives. Il semble que la prise d'eau de l'usine entraînait de l'eau et des microcrustacés de l'interface eau-sédiments. Les filets à plancton utilisés à la verticale ne permettant pas d'effectuer un prélèvement efficace dans le premier mètre inférieur de la colonne d'eau, les échantillons des prises d'eau pourraient bien représenter plus exactement la structure des communautés de copépodes et de cladocères de la zone riveraine que les échantillons obtenus par les méthodes classiques.

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Detection of changes in chemical characteristics and/or community structure of a water body is limited by study design and the inherent variability of the system. Temporal variability (seasonal, between years) often is the major source of variance necessitating frequent sampling to detect long-term trends (Evans and Sell 1983). However, it is not practical to conduct temporally intensive studies over large water bodies where high costs of vessel operation, poor weather, and ice cover preclude frequent sampling. Sampling from a limited number of representative, fixed locations such as water intakes is an alternative method for investigating long-term trends.

For the Great Lakes, sampling at municipal intakes provided detailed evidence of changes in the chemical characteristics of these lakes (Beeton 1969). Intake sampling, which documented increasing phytoplankton standing stocks between the 1930s and 1960s as a result of cultural eutrophication (Damann 1960; Davis 1964), now is documenting decreasing phytoplankton standing stocks as a result of remedial actions (Nicholls et al. 1977; Danforth and Ginsberg 1980). However, intake sampling has not been utilized to investigate long-term trends in zooplankton populations. We report the results of a study investigating the representativeness of an intake (Donald C. Cook Nuclear Plant) as a fixed sampling location for inshore region zooplankton.

Materials and Methods

Lake Sampling

Lake surveys were conducted once a month. Stations between the 5- and 10-m depth contour provided the inshore region data base (Fig. 1). Major surveys, consisting of 13 stations, were conducted in April, July, and October. Short surveys consisting of seven stations were sampled during May, June, August, September, November, and, on occasion, December.

Zooplankton were collected at each station with a 50-cm, 156-μm mesh net equipped with a calibrated flowmeter and

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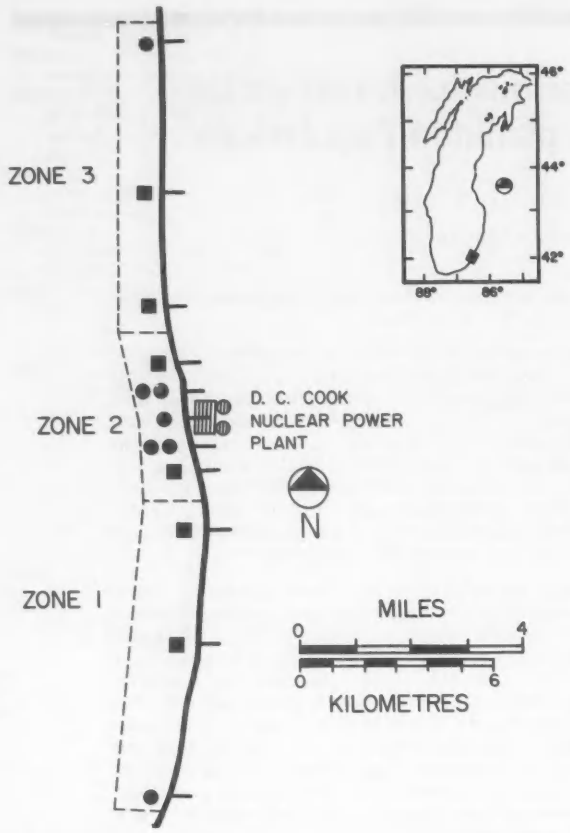

FIG. 1. Study area showing stations that were sampled monthly during April–October (●) or only in April, July, and October (■). The station immediately offshore of the power plant is centered over the discharge jets. See text for further discussion of intake and discharge structures.

hauled from approximately 1 m off the bottom to the lake surface. Two replicate samples were collected at each station.

Intake Sampling

At full operational capacity, the Donald C. Cook Nuclear Plant (Fig. 1) once-through cooling system utilizes approximately 6300 m^3/min. Cooling water is drawn through three intake pipes located approximately at the 7.3-m depth contour. Water enters a large, 9-m deep by 50-m long intake forebay before passing through the condenser cooling systems of this two-unit plant (United States Atomic Energy Commission 1973).

Entrainment samples were collected once a month from February 1975 to December 1981. During the cruise season, entrainment sampling was conducted within 1 or 2 d of a cruise. Samples in the intake forebay were collected from a depth of 5.5 m from a location determined to be statistically representative of the forebay (Evans et al. 1982).

Two replicate 5-min samples (approximately 1 m^3 each) were collected at sunset, midnight, sunrise, and noon. A Hale diaphragm pump withdrew water from the forebay and discharged it into a 20-cm (February 1975 to July 1977) or 30-cm diameter (after July 1977), 156-μm mesh net that was suspended in a barrel of water.

Laboratory Methods

In the laboratory, each sample was subdivided in a Folsom plankton splitter as many times as necessary to give two subsamples of 350–500 organisms each. Adult copepods and cladocerans were identified to species and immature copepodites to genus; nauplii were combined as a group. Further information on collection and laboratory techniques, overall study design, and history and an evaluation of the effects of power plant operation on zooplankton populations can be found in Evans et al. (1982).

Statistical Analyses

We examined data collected between 1975 and 1981 to determine whether intake sampling provided abundance estimates that were similar to those obtained by directly sampling the inshore region. We calculated the correlation (linear coefficient of correlation) between the 54 concurrent intake and inshore region observations. Various combinations of data were analyzed (see next paragraph). Analysis of variance (one-way, fixed effects) was used to determine whether intake abundance estimates differed significantly ($\alpha = 0.05$) from inshore region abundance estimates for the same 54 observation data sets. Finally, we calculated the ratio (R_g) of the geometric mean ($n = 54$) of zooplankton densities in the inshore region to the geometric mean of densities in the intake samples.

Four combinations of data were analyzed: (1) mean intake abundance ($n = 4$ diel observations) with the mean abundance of zooplankton in the inshore region ($n = 7$ or 13 stations; Fig. 1), (2) mean intake abundance ($n = 4$ diel observations) with mean abundance in zone 2 ($n = 5$ or 7 stations) in the immediate vicinity of the plant, (3) noon intake abundance ($n = 1$) with the mean abundance in the inshore region ($n = 7$ or 13 stations), and (4) noon intake abundance ($n = 1$) with the mean abundance in zone 2 ($n = 5$ or 7 stations).

The untransformed data did not meet the assumptions of normality. Log-transformed data did meet these assumptions; therefore, statistical analyses were performed on log-transformed data.

Results

Zooplankton abundances in the inshore region (zones 1–3) exhibited strong seasonal variation from cruise to cruise. Zooplankton standing stocks (Fig. 2, 3, 4) were low in spring and attained maxima in summer and autumn. There was a large decrease in standing stocks between the last cruise of a year and the April cruise of the following year. Zooplankton collected by noon intake sampling (Fig. 2, 3, 4) exhibited similar abundance patterns (seasonal, annual) as zooplankton collected in the inshore region.

Some consistent differences were noted in taxa abundances between the intake and inshore region. Nauplii tended to occur in lower concentrations in intake samples between 1975 and 1977 when a smaller diameter net was used. Adult *Cyclops bicuspidatus thomasi* generally occurred in higher concentrations in the intake series than in inshore region collections, especially during the May–July period.

Noon intake abundances generally were highly correlated ($r > +0.65$) with zooplankton abundances in zone 2, the area in

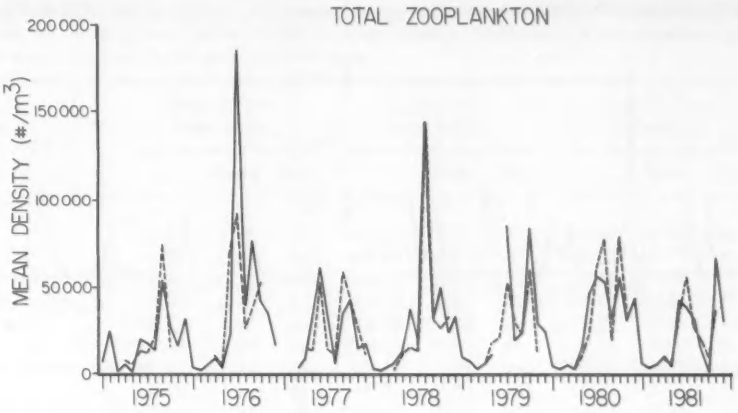

FIG. 2. Mean abundances of total zooplankton in inshore region (---) (zones 1–3) and in noon intake (—) samples.

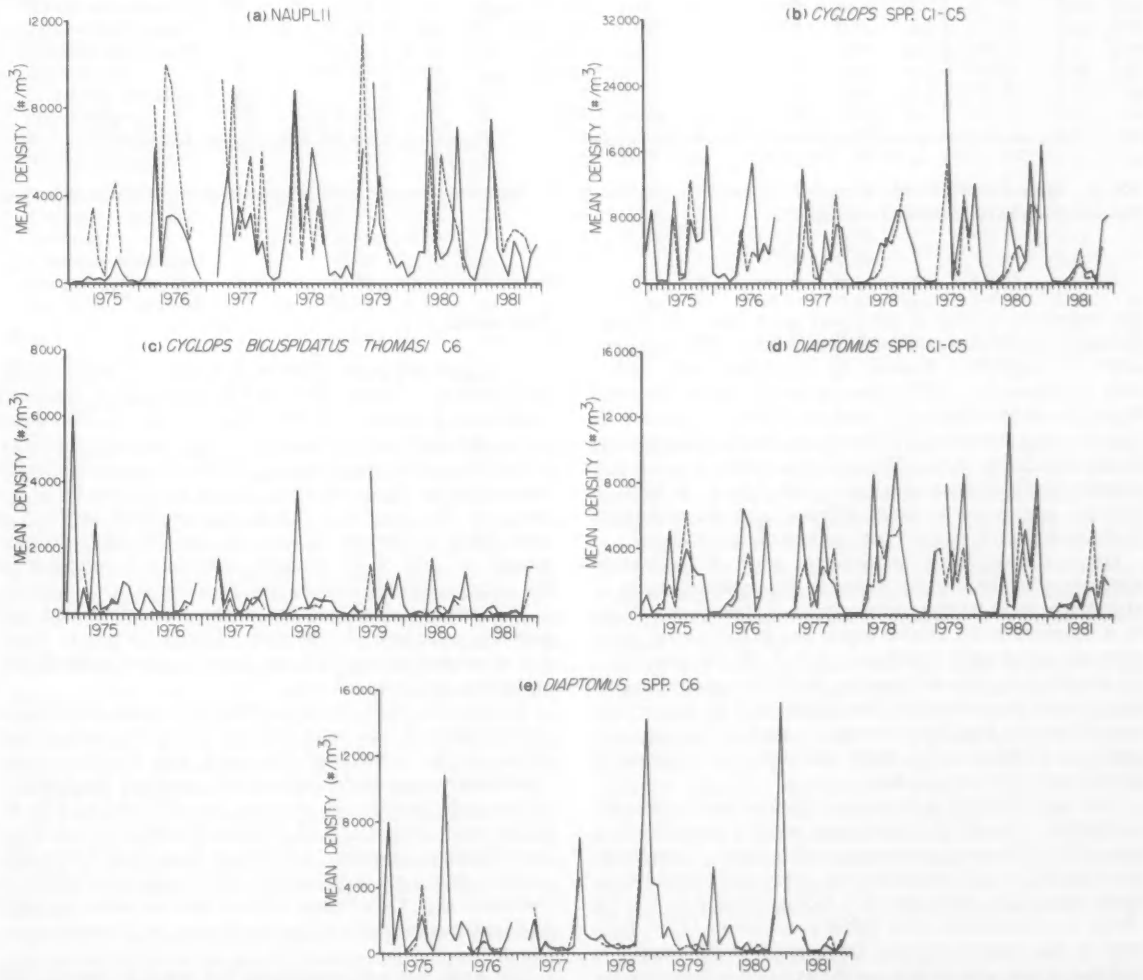

FIG. 3. Mean abundances of (a) nauplii, (b) *Cyclops* spp. C1-C5, (c) *C. bicuspidatus thomasi* C6, (d) *Diaptomus* spp. C1-C6, and (e) *Diaptomus* spp. C6 in inshore region (---) (zones 1–3) and in noon intake (—) samples.

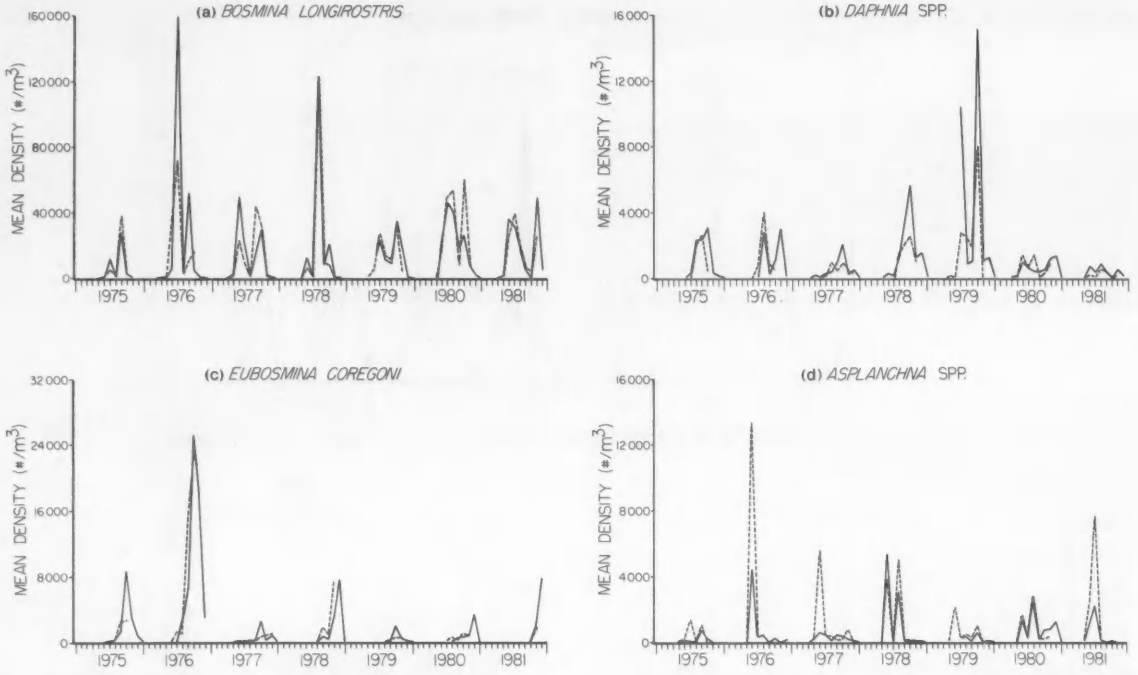

FIG. 4. Mean abundances of (a) *Bosmina longirostris*, (b) *Daphnia* spp., (c) *Eubosmina coregoni*, and (d) *Asplanchna* spp. in inshore region (---) (zones 1–3) and in noon intake (—) samples.

the immediate vicinity of the power plant (Table 1). Lower correlations were observed for nauplii, adult *Cyclops* spp., and adult *C. bicuspidatus thomasi*. All correlations were statistically significant ($p < 0.05$). Noon intake abundances also were highly correlated with zooplankton abundances in zones 1–3, i.e. the region extending 11 km north and south of the plant site. Intake abundances (mean of four diel observations) were highly correlated with abundances in zone 2 and zones 1–3 (Table 1). Overall, there were no large differences in the correlation coefficients for the various combinations of data analyzed.

For most taxa, R_g of the geometric mean of zooplankton abundances in the inshore region to the geometric mean of abundances in the intake approximated 1. Similarly, differences in abundances in the inshore region and in the cooling water were not statistically significant ($p < 0.05$) for most taxa. Consistent exceptions were nauplii, adult *Cyclops* spp., and *C. bicuspidatus thomasi* adults. Diel variations in abundance were not statistically significant (month × time of day; one-way analysis of variance) for most taxa with the exception of *Eurytemora affinis* copepodites.

Rare taxa observed in the intake waters included epibenthic and benthic copepods and cladocerans, which accounted for less than 0.5% of the microcrustacean standing stocks. Correlations between intake and inshore region sampling for these taxa, while statistically significant ($p < 0.05$), were low ($r < 0.50$) (Table 1). Abundances were higher in the intake ($R_g < 1.00$) than in the inshore region (Table 1). Diel differences in abundance were statistically significant only for *Eurycerus* sp. and *C. vernalis* copepods.

Discussion

We suggest that power plant intakes can serve as representative sampling locations for investigating long-term trends in zooplankton populations in the inshore region of various water bodies; the usefulness of intakes for long-term water chemistry and phytoplankton studies already has been demonstrated and is summarized in the introduction. By the criteria we used, the intake of the Donald C. Cook Nuclear Plant provided a representative estimate of seasonal and annual abundance patterns of most of the common euplankton zooplankton in the inshore region. Correlations between taxa abundances in the inshore region and the intake were significant and generally were high. Furthermore, differences in taxa abundances between the two locations generally were not significant; exceptions are discussed later.

Whereas our study was conducted at an intake that utilizes large volumes of lake water (6300 m^3/min), other intakes that utilize smaller volumes of lake water may provide equally representative population estimates of nearshore zooplankton. For example, preliminary results of a study conducted by B. Manny and R. Heberger (U.S. Fish and Wildlife Service, Great Lakes Fishery Laboratory, 1451 Green Road, Ann Arbor, MI, unpubl. rep.) at a low-velocity (1.33 m^3/min) water intake in Hammond Bay, Lake Huron, indicate that the intake provided representative estimates of bay crustacean zooplankton populations.

Our study has not investigated the optimum strategy for long-term studies using intakes. However, a number of general

TABLE 1. Correlations[a] between taxa abundance in inshore region and intake samples for the 54 concurrent observations over the 1975–81 period. Also shown is the ratio of the geometric means of inshore:intake densities. *Differences in taxa abundance between the intake and inshore region were significant at $\alpha = 0.05$; ns = nonsignificant differences.

Taxon	Zones 1–3 vs noon intake			Zone 2 vs noon intake			Zones 1–3 vs all intakes			Zone 2 vs all intakes		
	r	Ratio	Sig.	*r*	Ratio	Sig.	*r*	Ratio	Sig.	*r*	Ratio	Sig.
Euplankton												
Nauplii	+0.61	1.51	*	+0.58	1.52	*	+0.64	1.36	ns	+0.61	1.38	*
Cyclops spp. C1–C5	+0.87	0.79	ns	+0.86	0.85	ns	+0.85	0.72	ns	+0.84	0.77	ns
Cyclops spp. C6	+0.46	0.51	ns	+0.45	0.52	ns	+0.38	0.31	*	+0.41	0.32	*
Cyclops bicuspidatus thomasi C6	+0.48	0.60	ns	+0.48	0.62	ns	+0.42	0.42	*	+0.43	0.44	*
Tropocyclops sp.[b] C1–C6	+0.89	1.37	ns	+0.89	1.28	ns	+0.93	0.86	ns	+0.92	0.81	ns
Diaptomus spp. C1–C5	+0.78	0.96	ns	+0.78	1.04	ns	+0.78	0.90	ns	+0.78	0.98	ns
Diaptomus spp. C6	+0.77	1.41	ns	+0.79	1.39	ns	+0.85	1.05	ns	+0.86	1.04	ns
D. ashlandi C6	+0.70	1.67	ns	+0.72	1.63	ns	+0.73	1.04	ns	+0.75	1.01	ns
D. minutus C6	+0.66	1.77	ns	+0.67	1.68	ns	+0.71	1.24	ns	+0.71	1.18	ns
D. oregonensis C6	+0.73	1.28	ns	+0.72	1.31	ns	+0.77	0.86	ns	+0.76	0.88	ns
D. sicilis C6	+0.86	1.08	ns	+0.85	1.15	ns	+0.88	0.88	ns	+0.88	0.94	ns
Epischura lacustris C1–C6	+0.83	0.96	ns	+0.82	1.02	ns	+0.90	0.89	ns	+0.89	0.87	ns
Eurytemora affinis C1–C6	+0.89	1.11	ns	+0.89	1.01	ns	+0.95	0.68	ns	+0.94	0.61	ns
Limnocalanus macrurus C1–C6	+0.90	1.04	ns	+0.90	1.04	ns	+0.94	0.61	ns	+0.94	0.61	ns
Bosmina longirostris	+0.96	1.10	ns	+0.96	1.02	ns	+0.97	0.99	ns	+0.96	0.92	ns
Daphnia spp.	+0.90	0.78	ns	+0.89	0.77	ns	+0.89	0.65	ns	+0.87	0.64	ns
D. galeata mendotae	+0.73	1.18	ns	+0.73	1.12	ns	+0.76	0.80	ns	+0.75	0.76	ns
D. retrocurva	+0.89	0.72	ns	+0.88	0.76	ns	+0.88	0.64	ns	+0.87	0.68	ns
Eubosmina coregoni	+0.89	1.83	ns	+0.88	1.74	ns	+0.93	1.22	ns	+0.93	1.16	ns
Asplanchna spp.	+0.92	1.76	ns	+0.92	1.67	ns	+0.94	1.37	ns	+0.94	1.30	ns
Epibenthos/benthos												
Cyclops vernalis C6	+0.43	0.34	*	+0.44	0.29	*	+0.48	0.11	*	+0.47	0.09	*
Alona spp.	+0.25	0.60	*	+0.18	0.59	*	+0.41	0.28	*	+0.33	0.27	*
Chydorus sphaericus	+0.49	0.38	*	+0.50	0.38	*	+0.50	0.20	*	+0.49	0.20	*
Eurycercus lamellatus	+0.38	0.43	*	+0.36	0.42	*	+0.48	0.15	*	+0.45	0.15	*
Macrothrix laticornis	+0.43	1.01	ns	+0.37	1.00	ns	+0.31	0.84	ns	+0.28	0.83	ns
Total	+0.86	0.95	ns	+0.83	0.97	ns	+0.85	0.90	ns	+0.83	0.91	ns

[a]df = 53; for $\alpha = 0.05$, $r = 0.27$; for $\alpha = 0.01$, $r = 0.34$.
[b]*T. prasinus mexicanus*.

statements can be made. First, intake sampling may require less sampling effort than lake surveys to obtain similar levels of precision in population estimates for a given region. For the seven inshore region stations that were sampled consistently (Fig. 1) between April and October, station accounted for 1.3% (1976) to 14.6% (1977) of the variance (month × station analysis of variance, Evans and Sell 1983). Conversely, for similar analyses of the intake data (where "station" = time of day), "station" accounted for less than 1% of the variance (M.S. Evans, unpubl. data). This implies that a smaller number of intake samples would provide as precise a population estimate as is obtained by sampling a greater number of stations in the inshore region. Mixing in the intake pipes and in the forebay may provide a more homogeneous sample than in the lake where zooplankton patches exist (Evans and Sell 1983).

Diel variations in abundances were not statistically significant for most taxa. Therefore, we suggest that a single midday sample is sufficient to estimate the abundances of most of the common euplanktonic crustacean zooplankton in the inshore region.

Finally, three cases (nauplii, adults *Cyclops* spp., epibenthic and benthic microcrustaceans) of lower correspondence between intake and inshore region abundance estimates warrant further discussion. Nauplii occurred in relatively low concentrations in the intake samples between 1975 and 1977 when a smaller diameter plankton net was used. Significant numbers of nauplii probably were lost in intake collections when the pump forced small nauplii through the meshes of the 156-μm mesh net. This loss was reduced by increasing net diameter, which increased the distance the discharged water traveled before zooplankton were impinged on the net, i.e. it reduced flow velocity at the net meshes. Future studies should be designed to minimize the loss of small zooplankton.

There was strong evidence that the intake entrained water from the vicinity of the sediments, i.e. the lower 1 m of the water column. For example, after 1977, nauplii abundances were sometimes much greater in the intake than in the inshore region, e.g. August 1979. Such nauplii probably originated from the epibenthic and benthic copepod communities. Similarly high concentrations of adult *Cyclops bicuspidatus thomasi* may be related to entrainment. Whereas adult *C. bicuspidatus thomasi* are a common constituent of Lake Michigan zooplankton (Gannon 1975; Evans et al. 1980), they also are abundant at the sediment–water interface (Evans and Stewart 1977; Evans 1984).

Other epibenthic and benthic taxa were rare in inshore region collections but were more frequently collected and in greater abundances in intake samples. Significantly higher intake

densities do not appear to be due to elevated abundances of such organisms in the riprap area protecting the intake pipes; we observed that only a few such taxa occurred in apparently higher densities in the vicinity of the plant intakes (Evans 1984). The more important factor appears to be the greater susceptibility of epibenthic and benthic taxa to entrainment than to capture by the vertically hauled plankton net in the inshore region. However, intake sampling may provide a more accurate representation of inshore region copepod and cladoceran community structure than traditionally employed methods for sampling these waters. A final advantage to intake sampling is that it provides the opportunity to sample the inshore region at relatively low cost and during time periods (especially winter) when inclement weather prevents safe ship operation.

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Comparison of *N,N*-Dimethylformamide, Dimethyl Sulfoxide, and Acetone for Extraction of Phytoplankton Chlorophyll

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Speziale, B. J., S. P. Schreiner, P. A. Giammatteo, and J. E. Schindler. 1984. Comparison of *N,N*-dimethylformamide, dimethyl sulfoxide, and acetone for extraction of phytoplankton chlorophyll. Can. J. Fish. Aquat. Sci. 41: 1519–1522.

We evaluated the suitability of *N,N*-dimethylformamide (DMF) for extraction and measurement of chlorophyll in phytoplankton and compared the extraction efficiencies of DMF, dimethyl sulfoxide (DMSO)–acetone, and 90% acetone. Absorbance spectra and specific absorption coefficients of chlorophylls *a*, *b*, and *c* in DMF and 90% acetone are similar. Acidification of 100% DMF extracts of pure chlorophyll *a* and healthy algal cultures produced acid ratios approximating 1.7. Thus, equations derived from the extinction coefficients of chlorophylls in 90% acetone can be applied to DMF extracts. DMF and DMSO–acetone each extract chlorophyll from green and blue-green algae more effectively than does 90% acetone. DMF is a better extractant than is DMSO–acetone for chlorococcalean species, especially when pigment concentrations are high, but the difference, although significant, is slight. The three solvents are equally efficient in extracting chlorophyll from samples composed of chrysophytes and flagellated chlorophytes. The lack of grinding when using either DMF or DMSO–acetone is a considerable convenience. The potential for destabilized spectrophotometer readings and contamination of the investigator due to the incomplete mixing of DMSO and acetone is eliminated when pure DMF is used.

On a évalué si le *N,N*-diméthylformamide (DMF) pouvait être utilisé pour l'extraction et le dosage de la chlorophylle dans le phytoplancton et on a comparé l'efficacité d'extraction du DMF, du mélange diméthyl sulfoxyde (DMSO)–acétone et de l'acétone à 90 %. Les spectres d'absorption et les coefficients d'absorption spécifique des chlorophylles *a*, *b* et *c* dans le DMF et l'acétone à 90 % sont semblables. L'acidification des extraits dans le DMF à 100 % de la chlorophylle *a* pure et de cultures d'algues saines ont donné des rapports d'environ 1,7. Ainsi, les équations dérivées des coefficients d'extinction des chlorophylles dans l'acétone à 90 % peuvent être appliquées aux extraits de DMF. L'efficacité d'extraction de la chlorophylle des algues vertes et des cyanophytes est supérieure dans le DMF et le mélange DSMO–acétone par rapport à l'acétone 90 %. On obtient une meilleure extraction avec le DMF pour les espèces de chlorococcales, notamment lorsque les concentrations de pigments sont élevées, mais la différence, quoique significative, est légère. L'efficacité d'extraction des trois solvants est la même dans le cas de la chlorophylle provenant d'échantillons composés de chrysothytes de chlorophytes flagellées. L'absence de broyage quand on utilise le DMF ou le DMSO–acétone constitue un avantage appréciable. Lorsqu'on utilise le DMF, on élimine la possibilité d'obtenir des lectures déstabilisées au spectrophotomètre et la possibilité de contamination par le chercheur si le mélange de DMSO et d'acétone est incomplet.

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Numerous techniques exist for the extraction and measurement of chlorophyll in algae. Acetone extraction (Parsons and Strickland 1963) is inefficient (Marker 1972; Stauffer et al. 1979) and dependent on the degree of cell lysis during the grinding process. Acetone extraction is particularly inapplicable to samples containing chlorococcalean algae, which are often difficult to lyse or extract. The chlorophyll extraction techniques using dimethyl sulfoxide (DMSO) alone (Hiscox and Israelstam 1979) or mixed with 90% acetone (Shoaf and Lium 1976; Burnison 1980) eliminate the need for grinding, since DMSO extracts intact cells. However, mixing DMSO and acetone can cause difficulties. The impermanent mixing and partial evaporation that results when the two solvents are combined produces "schlieren" within the cuvette and seeping of solvent to the outside of loosely capped test tubes or cuvettes. The swirling of solvents within the cuvettes can destabilize absorbance readings, and contamination of the user with the solvent is probable. *N,N*-Dimethylformamide (DMF), which also extracts intact cells, was used as an effective extractant of chlorophyll and protochlorophyll from etiolated cucumber seedlings (Moran and Porath 1980). Volk and Bishop (1968) used DMF to assay chlorophyll in the blue-green alga *Cyanidium* after methanol extraction proved to be nonquantitative. Methanol is a superior extractant to acetone (Talling and Driver 1963) but is very

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sensitive to changes in pH (Marker 1972, 1977). Stauffer et al. (1979) compared the extractive abilities of 90% acetone, methanol, and mixtures of DMSO–acetone and DMF–methanol. They recommended methanol or DMSO–acetone for extraction of cyanophytes but noted that methanol might be easier to work with. In that study, DMSO–acetone was found to be a superior extractant of green algae, consistent with the results of Shoaf and Lium (1976).

Our purpose was to assess the suitability of DMF for extraction and assay of algal chlorophyll concentrations and to compare the extractive abilities of DMF, 90% acetone, and DMSO–acetone. In addition, we wished to compare the spectral characteristics of chlorophylls *a*, *b*, and *c* in the three solvents with published spectra and to investigate the suitability of using DMF and DMSO–acetone extracts with the phaeophytin correction and trichromatic equations formulated from extinction coefficients of chlorophylls in 90% acetone.

Materials and Methods

Extinction Coefficients and Absorbance Spectra

Extinction coefficients of pure chlorophylls *a* and *b* (Sigma Nos. 5753 and 5878) in 99% mole pure DMF and 90% acetone were calculated by comparison with the reported extinction coefficients in diethyl ether. Approximately 1 mg of pure chlorophyll was dissolved in 4 mL of DMF. Aliquots of this stock solution were then added to 10 mL of diethyl ether, DMF, or 90% acetone. The concentration of DMF in the other solutions was only 2% and therefore its influence on the absorbance spectrum was considered to be negligible. Extinction coefficients in 90% acetone and DMF were determined for a series of six dilutions, for each of three batches of chlorophyll *a* and two batches of chlorophyll *b*.

Chlorophyll *c* was extracted from *Fucus* that had been immersed for 10 s in boiling seawater, immediately chilled in cold seawater, and damp-dried. The fronds were extracted in 100% acetone. This extract was transferred to hexane, washed with ice-cold 10% NaCl, and the hexane evaporated (T. Jacobsen, Rutgers University, Shellfish Research Laboratory, P.O. Box 587, Port Norris, NJ 08349, pers. comm.). The residue remaining after evaporation of the hexane was dissolved in ether prior to isolation of chlorophyll *c* on sugar–starch columns (Strain and Svec 1966, 1969) using the elution techniques of Jeffrey (1972), but without separation of the two component pigments, c_1 and c_2. Aliquots of a concentrated solution of the purified chlorophyll *c* in DMF were added to either 90% acetone, DMF, or DMSO–acetone in order to obtain absorbance spectra in each solvent. The concentration in DMF in each of the other solvents was 2.5% and was considered to have negligible effects on the observed spectra. Absorbances for all chlorophyll solutions were read in the region from 750 to 400 nm on a Hitachi 100-60 double beam spectrophotometer with a 3-nm bandpass.

Comparison of Extraction Efficiencies

Natural phytoplankton and cultured algae were collected on Whatman GF/C glass fiber filters (1.2 μm effective retention). Chlorophyll was extracted using the methods of Strickland and Parsons (1972) for the 90% acetone extracts and Burnison (1980) for the DMSO–acetone extracts. DMF extraction followed the technique of Burnison (1980), substituting DMF for both the DMSO and the added acetone. The filters were loosely rolled and placed in screw-cap vials to which enough DMF (approximately 4 mL) was added to cover the filter. The loosely capped vials were heated in a 65°C water bath for 10–15 min. After cooling, each vial was agitated and the contents poured into a graduated 15-mL centrifuge tube. The vial and filter were rinsed several times with DMF and the final volume adjusted to 10 mL with DMF. All extracts were cleared of cell debris and residual glass fibers by centrifugation (International Clinical Centrifuge, model CL, 3000 rpm, 2 min). Three or four replicate filters were extracted for each sample assayed. Absorbance was read at 750, 665, 664, 663, 648, 647, 645, and 630 nm in each of the three solvents. Each 10-mL extract was then acidified with 10 μL of concentrated HCl (Burnison 1980) and centrifuged again. Absorbance was read at 750 and 665 nm after acidification. The concentrations of chlorophyll *a* and phaeophytin *a* in the samples were calculated using the equations of Lorenzen (1967), but with 11.7 substituted for the 11.0 "absorption coefficient" (see Discussion). We used Statistical Analysis System (SAS) analysis of variance and paired Student's *t*-test procedures to compare the extraction efficiencies of the three solvents. We report the level of significance for each comparison.

TABLE 1. Specific absorption coefficients ($g^{-1} \cdot L^{-1}$) of chlorophylls *a* and *b* in DMF and 90% acetone.

Solvent	λ (nm)	Specific absorption coefficient	Reference
		Chlorophyll a	
DMF	664.5	83.89	Moran and Porath 1980
DMF	664	82.71[a]	Seely and Jensen 1965
DMF	665	72.114	Volk and Bishop 1968
DMF	665	85.48±3.96[b]	This paper
90% acetone	663	89.31	SCOR–UNESCO 1966
90% acetone	664.3	87.67	Jeffrey and Humphrey 1975
90% acetone	664	92.0	Parsons and Strickland 1963
90% acetone	664	91.1	Vernon 1960
90% acetone	663	71.0	Richards and Thompson 1952
90% acetone	662.0	85.73[a]	Seely and Jensen 1965
DMSO	665.9	78.79[a]	Seely and Jensen 1965
		Chlorophyll b	
DMF	648	51.69±2.23[b]	This paper
90% acetone	646.8	51.36	Jeffrey and Humphrey 1975
90% acetone	648	53.5	Parsons and Strickland 1963
90% acetone	648	52.5	Vernon 1960

[a]Calculated from the reported molar extinction coefficients.
[b]Mean specific absorption coefficient ± 1 SD.

Results

The absorbance spectra for purified chlorophylls *a* and *b* in DMF and in 90% acetone were very similar. The red band maximum for chlorophyll *a* was 665 nm in DMF and 664 nm in 90% acetone. The chlorophyll *b* maximum in DMF was at 648 nm. The specific absorption coefficient calculated for chlorophyll *a*, 85.5 ± 3.96 (SD), was similar to reported values in DMF and in 90% acetone (Table 1). The value for chlorophyll *b* in DMF, 51.7 ± 2.23, was comparable with those in 90% acetone.

The absorbance spectra for chlorophyll *c* in DMF, 90% acetone, and DMSO–acetone were indistinguishable. Maxi-

TABLE 2. Comparison of chlorophyll *a* and phaeophytin concentrations (calculated using the phaeophytin correction equations of Lorenzen 1967, substituting 11.7 for the 11.0 absorption coefficient) in algal cultures and lake samples extracted in DMF, 90% acetone, or DMSO–acetone. Data are averages of three or four replicates ± 1 SD.

	DMF	90% acetone	DMSO–acetone
Willard's Pond, Nov. 1982 ($\mu g \cdot L^{-1}$)[a]	36.1±0.9	40.5±2.1	35.4±0.4
Lake Hartwell, Nov. 1982 ($\mu g \cdot L^{-1}$)[b]	9.17±0.49	10.8±1.3	8.67±0.49
Lake Hartwell, Feb. 1983 ($\mu g \cdot L^{-1}$)	2.72±1.09	2.93±0.78	2.01±0.54
Lake Hartwell enriched with *Chlorella pyrenoidosa*, ($\mu g \cdot L^{-1}$)	96.0±4.3	72.3±5.4	87.4±1.9
Anabaena flos-aquae, ($mg \cdot L^{-1}$)	1.11±0.02	1.03±0.01	1.03±0.08
Scenedesmus quadricauda, ($mg \cdot L^{-1}$)	4.73±0.13	3.33±0.14	4.23±0.17

[a]Diatoms, *Trachelomonas*, *Gonyostomum*, *Mallomonas*, *Cryptomonas*.
[b]Diatoms, *Peridinium*, *Chlamydomonas*, *Scenedesmus*, *Ankistrodesmus*.

mum absorbance in the red region of the spectrum was at 631 and 582 nm, comparable with the 630.6/579.4 and 630.9/581.2 peaks of chlorophylls c_1 and c_2 in 90% acetone (Jeffrey 1972). Analysis of variance and *t*-test comparisons indicated that the specific absorption coefficients of chlorophyll *c* in DMF, DMSO–acetone, and 90% acetone were not significantly different ($P < 0.01$).

Acidification of purified chlorophyll *a* caused a shift in the red absorbance peak from 665 to 667 nm and a reduction in absorbance of 0.59 ± 0.02 compared with the absorbance before acidification. The acid ratio (A665/A665a) of pure chlorophyll *a* in DMF was 1.69 ± 0.07 ($n = 5$). Healthy *Scenedesmus* cultures extracted in DMF had a range of acid ratios from 1.64 to 1.73.

A comparison of the amount of chlorophyll extracted from various field and algal culture samples using DMF, 90% acetone, or DMSO–acetone is presented in Table 2. Chlorophyll *a* concentrations were calculated using the Lorenzen (1967) equation, with substitution of 11.7 for the 11.0 "absorption coefficient." Acetone was an effective extractant in the Lake Hartwell and Willard's Pond samples, which were dominated by chrysophytes and flagellates, but was significantly less efficient ($P < 0.01$), when compared using *t*-test analysis, for cultures containing the chlorococcalean green algae, *Scenedesmus* and *Chlorella*. DMF was a significantly better extractant ($P < 0.05$) than was DMSO–acetone for *Chlorella* cultures.

Discussion

Many diverse extinction coefficients for chlorophyll *a* have been recorded in the literature (Table 1). Unfortunately, evidence of the variability of the estimations is rarely presented. The specific absorption coefficient calculated for chlorophyll *a* in DMF is within the range of values reported for the pigment in 90% acetone or DMF. When we calculated the specific absorption coefficient for individual batches of pure chlorophyll *a* in DMF or 90% acetone, the values were identical. Thus, the apparent differences in reported coefficients are less the result of actually distinct spectra than of experimental variation and the various base values from which each coefficient is derived (see Talling and Driver 1963 for a discussion of pre-1961 values). The extinction coefficient of chlorophyll *a* in DMSO–acetone is also similar to values in 90% acetone (Shoaf and Lium 1976) although the calculated coefficients were not reported.

Since absorption coefficients and spectra of the three chlorophylls are similar in DMF, 90% acetone, and DMSO–acetone, equations derived for calculation of chlorophyll concentrations in 90% acetone solutions can be applied to extracts in the other solvents. The potential error incurred in using a set of acetone equations with extracts in the other solvents is less than that which occurs when different sets of equations are used to calculate chlorophyll concentration in a single acetone extract. In algal samples containing easily lysed cells, there is no significant difference at the 95% confidence level between concentrations of chlorophylls *a*, *b*, and *c* in DMF, 90% acetone, and DMSO–acetone extracts estimated using the Jeffrey and Humphrey (1975) equations. The differences in estimation of chlorophyll *a* in a single acetone extract, for samples having low concentrations of chlorophylls *b* and *c*, although affected by the intensity of absorbance (Chang and Rossman 1981), will be within 4% if the Jeffrey and Humphrey (1975), Parsons and Strickland (1963), or SCOR–UNESCO (1966) equations are used (Wartenberg 1978). However, trichromatic methods are extremely inaccurate in estimating concentrations of chlorophylls *b* and *c* (Rai 1973, 1980), especially in the presence of phycobilins (Stauffer et al. 1979) or phaeophytins (Moss 1967). Monochromatic equations such as that employed by Stauffer et al. (1979) or those methods that correct for phaeopigments (Lorenzen 1967; Moss 1967) are probably preferable, despite loss of information concerning chlorophylls *b* and *c*.

The equations for the estimation of concentrations of chlorophyll *a* and phaeophytin *a* in 90% acetone extracts (Lorenzen 1967) can be applied to DMF extracts, with substitution of 11.7 for the 11.0 "absorption coefficient." Lorenzen's absorption coefficient, similarly calculated by Moss (1967), is probably too low. Moss derived the 11.0 "proportionality factor" as the reciprocal of the chlorophyll *a* specific absorption coefficient of 90.8 $L \cdot g^{-1} \cdot cm^{-1}$ (Vernon 1960), which is 5% higher than the value more recently determined by Jeffrey and Humphrey (1975). Talling and Driver (1963) suggested a proportionality

factor of 11.9 for estimating the concentration of chlorophyll *a* based on its absorbance at 665 nm in a 90% acetone solution. Proportionality constants calculated from the chlorophyll *a* specific absorption coefficients listed in Table 1 range from 10.9 (Parsons and Strickland 1963) to 13.9 (Volk and Bishop 1968). The calculated specific absorption coefficients that we used in formulating the modified Lorenzen equations were not significantly different for 90% acetone and DMF extracts. Proportionality factors, calculated as the reciprocals of the derived absorption coefficients, were 11.7 for both solvents. Thus, 11.7 should be substituted for assays in both solvents and should be applied to DMSO–acetone extracts as well, since the chlorophyll *a* absorbance spectra are identical in 90% acetone and DMSO–acetone (Shoaf and Lium 1976; Hiscox and Israelstam 1979). The revised Lorenzen equations for calculation of chlorophyll *a* and pheophytin *a* in algal samples are as follows:

$$(1)\quad \text{Chl } a\ (\mu\text{g/L}) = \frac{11.7 \times 2.43 \times (665_0 - 665_a) \times v}{V \times l}$$

$$(2)\quad \text{Pheo } a\ (\mu\text{g/L}) = \frac{11.7 \times 2.43 \times ((1.7 \times 665_a) - 665_0) \times v}{V \times l}$$

where 665_0 = absorbance at 665 nm before acidification, 665_a = absorbance at 665 nm after acidification, v = total volume (millilitres) of solvent used for extraction, V = volume (litres) of water filtered, and l = path length (centimetres) of the cuvette.

The choice of extraction solvents should be based on the type and quantity of algae in the sample. Easily lysed algae such as chrysophytes and some flagellates are extracted as efficiently in 90% acetone as in DMF or DMSO–acetone. DMF is a better extractant than is DMSO–acetone for chlorococcalean algae, but the difference, although significant at the 0.05 level in a Student's *t*-test, is slight and is most pronounced in samples containing relatively high chlorophyll concentrations. The utility of the acetone extraction technique is reduced for natural phytoplankton samples where there is a potential for encountering algae that are difficult to lyse and are thus poorly extracted by grinding in 90% acetone. Thus, either DMF or DMSO–acetone is a preferable extractant for most phytoplankton samples.

The toxicity of the three solvents and the potential for contamination using each of the described methods must be considered in the decision to use DMF, DMSO, or 90% acetone for the extraction and measurement of chlorophyll in algal samples. Adverse physiological effects are known for prolonged exposure to acetone (Windholz 1976), DMF (Deichmann and Gerarde 1969), and DMSO (Kapp and Eventoff 1980). Shoaf and Lium (1976) cautioned that DMSO should be handled with caution despite its relatively low toxicity (LD_{50} in rats $> 20\ g \cdot kg^{-1}$ (Windholz 1976)) due to its ability to rapidly penetrate the skin. DMF is more toxic than is DMSO (LD_{50} in mice $= 1.122\ g \cdot kg^{-1}$ (Windholz 1976)) but is less likely to contaminate the user than is the DMSO–acetone mixture. The higher toxicity of DMF should be weighed against the greater likelihood of contamination using DMSO–acetone mixtures.

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NOTES

Effect of an Intertidal Mudflat on Plankton of the Overlying Water Column[1]

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The influence of a small tidal mudflat on the plankton of overlying waters was investigated by sampling flooding and ebbing waters. The mudflat depleted floodwaters of plankton during spring and summer. Removals of phytoplankton increased as the season progressed, indicating consumption as a function of activity of benthic filter feeders. Some zooplankton were removed from flooding waters in a similar seasonal pattern. We have demonstrated input of planktonic carbon to mudflats and indicated that mudflat organisms may consume significant portions of both primary and secondary planktonic production.

On a étudié, par le prélèvement d'échantillons d'eau du flux et du reflux, l'influence d'une petite laisse de vase sur le plancton des eaux la recouvrant. La laisse avait pour effet d'appauvrir les eaux en plancton au printemps et en été. La disparition du phytoplancton augmentait avec la saison, ce qui indique une consommation fonction de l'activité des organismes filtreurs du benthos. La disparition d'une partie du zooplancton des eaux du flux présentait la même allure saisonnière. Ces travaux démontrent l'existence d'un apport de carbone planctonique aux laisses de vase et indiquent que les organismes des laisses peuvent consommer des quantités appréciables de la production planctonique primaire et secondaire.

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Intertidal mudflats are depositional environments maintained in part by sediment modification activities of mudflat organisms. Because many mudflat organisms maintain themselves by filtering materials from overlying waters, interactions between mudflats and water columns may result in removal of suspended particulate materials, including phytoplankton, from the water. Such interactions have been observed for other shallow-water systems such as salt marshes (Woodwell et al. 1977) and coral reefs (Glynn 1973; Johannes and Gerber 1974), and there are strong suggestions that mudflat organisms have similar impacts on overlying waters. For example, extensive culture of some bivalves was implicated in lowered ambient concentrations of phytoplankton (Cadee and Hegeman 1974), Baillie and Welsh (1980) found benthic consumption of materials resuspended within tidal flats, and Wright et al. (1982) showed removal of phytoplankton from waters passing over a bed of mussels, *Mytilus edulis*. Measurements of benthic community respiration in intertidal areas (Es 1982) and estimates of phytoplankton biomass dynamics in shallow waters (Cloern 1982) resulted in a similar hypothesis: removal of phytoplankton to the benthos. However, in situ demonstration of phytoplankton consumption by mudflat communities has been difficult, primarily because it is often impossible to distinguish and sample only those portions of coastal waters that are directly influenced by mudflats. Another difficulty results from the shallow mudflat environments wherein warm temperatures, enhanced nutrient supply, or resuspension of benthic flora may influence planktonic biomass and where other feeding pressures on phytoplankton, especially by zooplankton, may obscure mudflat influences. Here, we report a study of a tidal cove whose drainage characteristics facilitated

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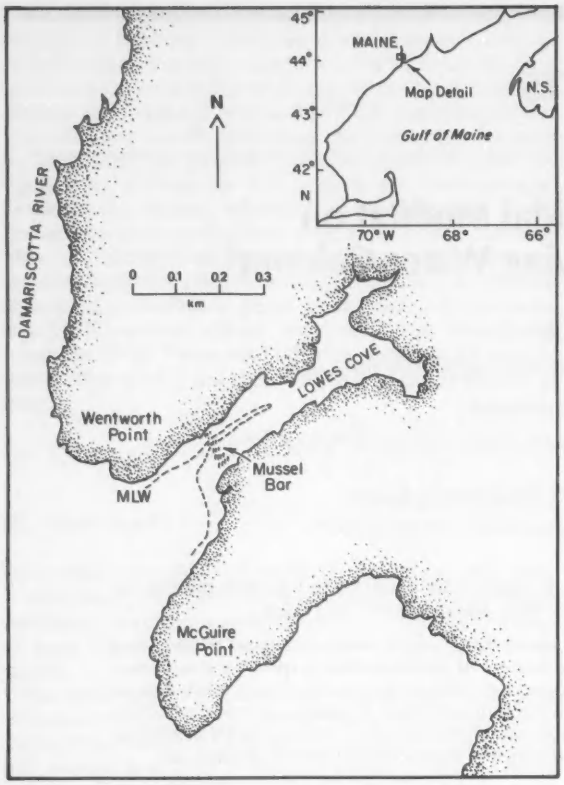

FIG. 1. Location and map of Lowes Cove. MLW = mean low water level.

identification of mudflat-influenced water. We monitored both phytoplankton and zooplankton in order to examine the effects of tidal incursions over mudflats on planktonic populations.

Sample Area and Methods

Our study was conducted at the entrance to Lowes Cove, a 5.9-ha tidal cove of the Damariscotta River estuary in central Maine (Fig. 1). The cove has a narrow entrance guarded by rocky headlands and a shoal mussel bar. The mean tidal range within the cove is slightly less than 3 m and currents in the tidal channel average nearly 0.2 $m \cdot s^{-1}$ (Anderson and Mayer 1984). Drainage is complete landward of the mussel bar on each tide; water in the shallow area protected by Wentworth and McGuire points represents alternately incoming estuarine water or outgoing cove water. Water temperatures in the cove have a seasonal range from <0 to >25°C, with maximia slightly greater than estuarine surface waters. Salinities are essentially those of coastal waters. Hydrographic, topographic, and sedimentological details for Lowes Cove are given by Anderson et al. (1981).

Sampling coincided approximately with water temperatures of 0, 5, 10, and 20°C during the first half of 1980. Samples were collected immediately outside the mussel bar in a shallow draft boat. On the first sampling date a 50-cm-diameter 165-μm-mesh plankton net was towed behind the boat. To avoid dragging the net in such shallow water and to ensure complete collection of smaller zooplankton, we collected subsequent samples with a 30-cm-diameter 80-μm-mesh net held vertically in front of the bow of the boat. We took 10-min tows 3 h before and 3 h after high tides. The shallow cove entrance was completely circled several times during a 10-min tow, thus ensuring representative samples of the plankton. Whole water samples were collected using 2.5-L Van Dorn bottles at the start and finish of each tow. Aliquots were taken from each water sample for chlorophyll *a* (Chl *a*) assays and for phytoplankton cell counts and identifications. Replicate Chl *a* samples were collected on Whatman GF/C filters, measured fluorimetrically following Strickland and Parsons (1972), and reported as means of pre- and post-tow values. Individual data in all cases were within 12% of the means, and were generally within 5%. Phytoplankton were preserved in Lugol's solution, settled in 10-mL chambers, and examined with an inverted microscope. Net samples were preserved in 5% buffered formalin and settled overnight in Imhoff cones to determine settled volume biomass. Each sample was then diluted to 10 times the settled volume, and all zooplankton organisms in 1-mL aliquots were identified and counted using Sedgwick–Rafter cells.

Results

As the season progressed, progressively greater amounts of Chl *a* were removed from waters flooding the mudflat (Fig. 2). In February, with water temperatures <0°C, Chl *a* concentrations were actually greater in waters draining from the mudflat than in those flooding it. At 5°C (April), a slight removal of Chl *a* during flood tide was evident, even though both flood and ebb concentrations were lower than those of the February bloom. In May and July, with water temperatures >10°C,

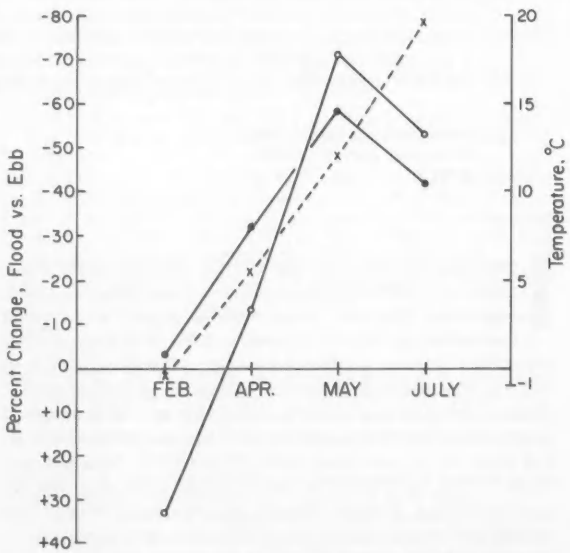

FIG. 2. Percentage changes of phytoplankton Chl *a* and zooplankton biomass in ebb vs. flood waters on each of four sampling dates in 1980. Water temperatures are included. Lines connecting data points are visual aids and do not necessarily represent conditions during intervals between actual sample dates. ●, zooplankton settled volume; ○, phytoplankton Chl *a*; ×, temperature.

TABLE 1. Phytoplankton cell counts (thousands of cells·L^{-1}) and Chl *a* concentrations on flood and ebb tides on each of four sample dates. Only dominant species are listed.

Species	Flood		Ebb	% Change
29 February 1980				
Thalassiosira nordenskioldii	172		166	−3
T. gravida	134		128	−4
Skeletonema costatum	38		28	−26
Detonula confervacea	26		14	−46
Chaetoceros debile	19		81	+326
(20 others)	14	(9 others)	17	+21
Total	403		434	+8
Chl *a* (μg·L^{-1})	10.0		15.0	+50
16 April 1980				
Cylindrotheca closterium	4		0.3	−92
(10 others)	3	(9 others)	4	+33
Total	7		4	−43
Chl *a* (μg·L^{-1})	1.8		1.6	−14
29 May 1980				
Skeletonema costatum	411		5	−99
Cylindrotheca closterium	5		—	−100
Thalassionema nitzschoides	3		2	−33
Detonula confervacea	3		—	−100
(7 others)	3	(4 others)	4	+33
Total	425		11	−97
Chl *a* (μg·L^{-1})	3.8		1.1	−71
28 July 1980				
Nitzschia delicatissima	140		31	−78
Rhizosolenia delicatula	33		5	−85
Ditylum brightwelli	15		1	−93
Cylindrotheca closterium	10		7	−30
Navicula spp.	—		4	—
(9 others)	6	(6 others)	2	−67
Total	204		50	−76
Chl *a* (μg·L^{-1})	4.0		1.9	−53

relatively large removals of Chl *a* occurred, amounting to 50–75% of incoming water values.

Pigment measurements were supported by cell count data (Table 1). Numbers of phytoplankton in waters draining from the cove showed a slight increase over flooding waters in February, a small decrease in April, and larger decreases in May and July. Species of *Thalassiosira* were dominant in February, typical of late winter–early spring blooms in the Damariscotta (Cura 1981). The slight decrease in cell numbers in April was primarily seen in the diatom *Cylindrotheca costerium*; total numbers were very low in flood and ebb samples. In May and July, single species (*Skeletonema costatum* and *Nitzschia delicatissima*, respectively) dominated the flooding waters, and it was removal of these that accounted for essentially all of the decreases observed in ebbing waters. Removal in May was notably efficient, with 97% of total phytoplankton and 99% of the dominant organism removed from the water. There was little evidence of selective removal of specific sizes, types, or species of phytoplankton: large cylindrical cells (e.g. *Ditylum brightwelli*), small chain-forming cells (*Skeletonema costatum*), and pennate cells (*Nitzschia delicatissima*) were all removed.

Seasonal removal of water column phytoplankton in Lowes Cove was also evident in preceding years. On 20 dates in April through November of 1978, Chl *a* was measured at the mouth of Lowes Cove and at a midchannel station in samples collected without regard to stage of tide. Subsequent segregation of the data according to tidal stage revealed a distinct pattern of seasonal phytoplankton removal (Fig. 3). There were no Chl *a* removals evident in waters leaving Lowes Cove in April, maximum removals in July, and no removals again in October. In contrast, Chl *a* concentrations in samples of Lowes Cove floodwaters were consistently similar to concentrations in midchannel samples. In a series of samples collected every 4 h for 24 h at the same two locations, Lowes Cove and midchannel, during 1979 (Fig. 4), Chl *a* concentration in waters draining the cove were always lower than midchannel concentrations, while flood tide concentrations were equal to or slightly larger than

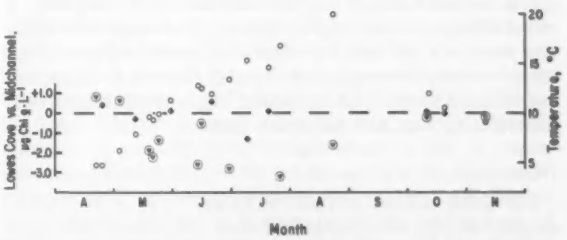

FIG. 3. Differences in Chl *a* concentration between Lowes Cove and a midchannel location in 1978. ▼, ebb samples collected from high water to 1 h before low tide; ◆, flood samples, low water to 1 h before high; ○, water temperatures. Absolute values were 1.7–4.7 μg Chl *a*·L^{-1} at Lowes Cove and 1.5–6.1 μg Chl *a*·L^{-1} in midchannel.

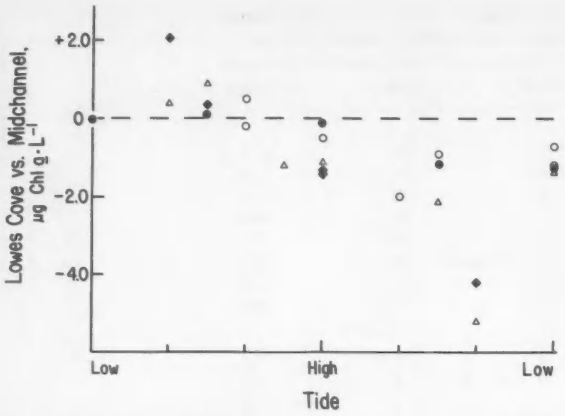

FIG. 4. Differences in Chl *a* concentration between Lowes Cove and a midchannel location during tidal cycles. Samples were collected every 4 h for 24 h in 1979. ●, 14 June; ○, 20–21 June; ◆, 17–18 July; △, 8–9 August. Absolute concentrations were 1.2–8.3 µg Chl $a \cdot L^{-1}$ at Lowes Cove and 1.1–10.2 µg Chl $a \cdot L^{-1}$ at midchannel.

midchannel concentrations. Removals were evident in both diurnal and nocturnal samples. In addition, extent of removal in 1979, expressed as percentage of floodwater values, increased with increasing water temperatures from June to July and then decreased in August, mimicking the pattern observed in 1980.

Decreases in zooplankton biomass on ebb tides, determined from settled volumes, were evident on each 1980 sample data except in February (Fig. 2). Decreases were greatest in May and July, amounting to >40% of initial biomass. The volume decreases observed were attributable primarily to zooplankton. Phytoplankton volumes as percentages of settled volume in floodwaters, calculated using our cell numbers and specific and average cell volumes given by Lehman (1981), were 20, 1, 0.5, and 14% in February, April, May, and July samples, respectively, so that complete removal of floodwater phytoplankton would have influenced settled volumes by no more than those percentages. Decreases in zooplankton biomass were accompanied by corresponding decreases in numbers of individual organisms (Table 2). Differences in total numbers between flood and ebb samples may not be significant according to confidence limits calculated for single tows at single stations (Lee and McAlice 1979), but by the same criteria, significant differences were evident in numbers of the most abundant zooplankters and it was the disappearance of these that resulted in seasonal removal patterns. These numerically dominant species included straight hinge bivalve larvae (May and July), a rotifer (*Synchaeta* sp.) (April), and naupliar stages of *Acartia* spp. copepods (all months). Removals were evident without regard to size, developmental stage, or taxonomic distinction and included nauplii and copepodite stages of many copepods, tintinnids, rotifers, and polychaete larvae.

Discussion

The small shallow entrance to Lowes Cove enabled us to sample relatively large portions of waters alternately flooding or draining the cove. We are confident therefore that removals of phytoplankton we observed resulted from processes occurring within the cove, most likely consumption of the phytoplankton by mudflat organisms. Other removal processes that might be

TABLE 2. Zooplankton counts (thousands of organisms $\cdot m^{-3}$) and settled volumes on flood and ebb tides on each of four sample dates. Only organisms with abundances $\geq 10^3 \cdot m^{-3}$ in flood or ebb samples are included.

Species	Flood	Ebb	% Change
29 February 1980			
Acartia sp. NI–NIII	1	7	+587
Acartia sp. NIV–NVI	4	3	−22
Acartia sp. CI–CIII	2	1	−55
Acartia longiremis adults	—	2	—
Eurytemora herdmani NI–NIII	1	2	+93
Oithona similis NI–NIII	—	3	—
O. similis NIV–NVI	—	1	—
O. similis CI–CIII	—	2	—
O. similis CIV–CV	—	2	—
O. similis adults	3	7	+116
Pseudocalanus minutus NI–NIII	—	5	—
P. minutus NIV–NVI	4	8	+107
P. minutus CI–CIII	—	2	—
P. minutus CIV–CV	3	1	−65
P. minutus adults	2	1	−44
Temora longicornis adults	2	3	+32
Balanus sp. NI–NIII	274	129	−53
Balanus sp. NIV–NVI	191	223	+17
Hansen's nauplius	2	<1	−80
Polychaete trochophores	12	1	−89
Tintinnopsis sp.	—	1	—
Total	504	406	−19
Settled volume (mL $\cdot m^{-3}$)	2.47	2.41	−2
16 April 1980			
Acartia sp. NI–NIII	9	2	−82
Acartia sp. NIV–NVI	7	2	−70
Temora longicornis NI–NIII	1	<1	−60
Copepod eggs	1	<1	−58
Synchaeta sp.	17	6	−63
Total	35	11	−69
Settled volume (mL $\cdot m^{-3}$)	0.71	0.48	−32
29 May 1980			
Acartia sp. NI–NIII	23	17	−27
Acartia sp. NIV–NVI	14	15	+10
Acartia sp. CI–CIII	2	1	−67
Acartia sp. CIV–CV	2	1	−58
Eurytemora herdmani NIV–NVI	1	<1	−77
Temora longicornis NI–NIII	2	2	+41
T. longicornis NIV–NVI	1	1	0
Bivalve larvae	24	10	−60
Polydora ligni larvae	2	1	−62
Unid. invertebrate eggs	—	5	—
Total	72	53	−26
Settled volume (mL $\cdot m^{-3}$)	2.58	1.07	−58
28 July 1980			
Acartia sp. NI–NIII	2	4	+88
Acartia sp. NIV–NVI	15	5	−65
Acartia sp. CI–CIII	3	<1	−93
Acartia sp. CIV–CV	2	<1	−67
Eurytemora herdmani NIV–NVI	<1	1	+200
E. herdmani CI–CIII	1	<1	−58
Temora longicornis NI–NIII	1	4	+185
T. longicornis NIV–NVI	2	<1	−73
Unid. Harpacticoids CIV–CV	1	2	+50
Flavella sp.	11	3	−70
Tintinnopsis sp.	6	3	−48
Bivalve larvae	22	17	−24
Synchaeta sp.	6	4	−25
Neries succinea larvae	1	—	−100
Total	74	46	−39
Settled volume (mL $\cdot m^{-3}$)	1.45	0.84	−42

invoked do not appear to conform to the reproducible patterns observed.

Removal of phytoplankton by settling was not likely to have been effective in a tidally dominated regime such as Lowes Cove. Instead, there was resuspension of surface sediments within the cove, with resuspended materials a much larger fraction of total suspended particulate matter in the spring (>90%) than in the winter (Anderson and Mayer 1984). Although seasonal alterations in textural characteristics of surface sediments may influence seasonal resuspension patterns, it is clear that turbulence sufficient to induce resuspension precludes settling. More important, Chl *a* did not accumulate in surface sediments of Lowes Cove during the months we observed removals (Rahaim 1980), indicating that intact cells were not settling from water columns. In addition, settling from surface waters at the Lowes Cove entrance should not have been different than at the midchannel station <300 m distant.

Zooplankton were evidently not responsible for observed phytoplankton removals. In our 1980 samples, largest numbers of zooplankton, primarily active and voracious barnacle nauplii, occurred in February without corresponding decreases in phytoplankton. In the first 6 mo of both 1979 and 1980, maximum abundances of zooplankton and ichthyoplankton in the Damariscotta estuary occurred in March (Townsend 1984), well before maximum phytoplankton removals were observed in Lowes Cove. Annually, largest numbers of zooplankton in the estuary, dominated by *Acartia* copepods, occurred in August and September (B. J. McAlice, unpubl. data), after maximum phytoplankton consumption in Lowes Cove. In addition, zooplankton biomass in these waters was inversely correlated with tidal height (Lee and McAlice 1979), which should have introduced a pattern opposite to that observed had zooplankton grazing been dominant in the cove. Zooplankton numbers also showed strong nocturnal increases in midchannel locations (Lee and McAlice 1979), but diel patterns in Lowes Cove did not show enhanced grazing. Finally, if phytoplankton consumption was due to zooplankton whose biomass and activity were simply patchy, consistent patterns such as described here would not have been evident.

We suggest that persistent seasonal patterns of removal of plankton from waters flooding Lowes Cove resulted from grazing by benthic organisms in the cove. The predominant suspension feeders in Lowes Cove are bivalves: blue mussels (*Mytilus edulis*) and soft-shelled clams (*Mya arenaria*) (Anderson et al. 1981). Filtration rates of these bivalves are known to be higher in late spring and summer months than in winter, due not only to changes in water temperatures (Schulte 1975; Walne 1972) but also to seasonal changes in food availability and reproductive activities (Widdows et al. 1979; Newell and Bayne 1980) and to an inability to acclimate completely to the extreme winter cold (Stickney 1964; Gilfillan et al. 1976; Eaton 1983). These bivalves are sufficiently numerous to filter significant fractions (>50%, W. Mook, unpubl. data) of the Lowes Cove tidal volume. Mussels in the shoal bar near the cove mouth are ideally situated to filter incoming waters, and Wright et al. (1982) have clearly demonstrated that such accumulations of mussels deplete overflowing waters of phytoplankton.

A seasonal grazing impact of Lowes Cove on its flooding waters is consistent with several other observations made within Lowes Cove. Rahaim (1980) found a seasonal pattern of phaeopigment concentrations in surface sediments, with low values March through May rising to higher values in June through September. This pattern was in contrast with relatively constant levels of Chl *a* in surface sediments during the same periods, and the phaeopigment accumulations were most evident in lower intertidal areas. Summer appearance of these chlorophyll degradation products, particularly in those areas that flood early in the tidal cycle, is certainly consistent with our observed summer maximum in phytoplankton removal. Total organic carbon and nitrogen in surface sediments increased over the entire cove from March to August, and the carbon isotopic composition of that organic material suggested planktonic sources (Mayer et al. 1984). Soft-shelled clams (*Mya arenaria*) in Lowes Cove showed maximum meat and shell growth from April to June in phase with seasonal removal patterns, and growth inside the mussel bar was less than growth outside (Newell 1982), consistent with depletion of food resources by the mussel bar. In addition, Anderson et al. (1981) described a seasonal deposition pattern for Lowes Cove with deposition dominant March through June and suggested biodeposition by suspension feeders as an important fraction of total deposition.

In all three years, consumption of phytoplankton within Lowes Cove diminished in mid- to late summer. Such a pattern is consistent with behavior of benthic suspension feeders, which can reduce feeding activities in response to nonoptimal suspended particulate concentrations, unpalatability of food materials, and life cycle changes. For example, the bivalve *Cardium edule* showed maximum ingestion in spring but feeding and metabolic rates declined after spawning (Newell and Bayne 1980). Raft-cultured mussels in the Damariscotta estuary showed similar patterns, with diminished growth rates and spawning in June (Incze et al. 1980). Analogous activity patterns among Lowes Cove benthic fauna would result in declines in phytoplankton consumption such as we observed. Alternately, in-cove benthic primary production or resuspension of organic deposition from spring feeding may have become increasingly important food sources for suspension feeders faced with diminishing summer standing crops of planktonic food.

Food materials ingested by bivalves in Lowes Cove can be as large as 35–50 μm (e.g. dinoflagellates such as *Gonyaulax* sp.); larger phytoplankton or zooplankton may be removed from water columns as pseudofecal materials. The apparent consumption of zooplankton, which occurred with a seasonal pattern strikingly similar to phytoplankton consumption, may thus also have been due to benthic grazing. Settlement of some bivalve larvae (May and July) may have contributed to the removals. For some crustaceans, notably barnacle larvae in February, molting in the cove could account for decreased numbers of an early developmental stage and increased numbers of subsequent stages in ebb waters.

The net influence of the mudflat as the season progressed was to decrease the amounts of plankton in floodwaters. Removals such as these provide the carbon pathway from plankton to benthos hypothesized by Es (1982) and Cloern (1982). We do not know whether resupply of plankton or other particulate materials to estuarine waters might occur in fall or winter erosional episodes nor can we yet demonstrate the direction or magnitude of net annual carbon flux. Clearly, significant proportions of planktonic primary producers, and perhaps of planktonic grazers as well, can be consumed by mudflat organisms. We emphasize suggestions of Officer et al. (1982) that in shallow waters, benthic consumption should be considered as an influence on phytoplankton. We extend those

suggestions to include consumption of zooplankton, and to areas of deeper water proximal to mudflats.

Acknowledgments

This work evolved from a course conducted by Dr B. J. McAlice, Ira C. Darling Center, Walpole, ME, and we dedicate the work to him. We also thank B. Mook, Ira C. Darling Center, for sharing unpublished data.

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Stress-Related Changes in Lake Whitefish (*Coregonus clupeaformis*) Associated with a Hydroelectric Control Structure

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Stress-related changes in lake whitefish (*Coregonus clupeaformis*) associated with a hydroelectric control structure. Can. J. Fish. Aquat. Sci. 41: 1528–1533.

Lake whitefish (*Coregonus clupeaformis*) that accumulate below the Lobstick control structure at the Churchill Falls hydroelectric facility exhibit many stress-related symptoms. Compared with fish from the reservoir or from a natural lake they score low on the following indices: liver somatic, gonad somatic, stomach content volumes, and condition factor; they have a low rate of circuli formation on the scales and they do not reproduce; proximate composition analyses identified reduced muscle protein levels and reduced ovarian protein and lipid. The liver may be the main site of lipid storage and the liver somatic index a sensitive condition indicator in whitefish.

Les corégones de lac (*Coregonus clupeaformis*) qui se groupent an aval de l'ouvrage de régularisation Lobstick des installations hydro-électriques de Churchill Falls présentent bon nombre de symptômes liés au stress. Comparativement aux poissons du réservoir ou d'un lac naturel, ces poissons présentent de faibles indices somatiques hépatiques et gonadiques, un volume stomacal peu élevé et un mauvais facteur de condition ; leur taux de formation des anneaux des écailles est peu important et ils ne se reproduisent pas. Des analyses grossières ont permis de noter des teneurs réduites en protéines musculaires et en protéines et lipides ovariens. Le foie peut être le principal point d'accumulation des lipides et l'indice somatique hépatique pourrait s'avérer un indicateur sensible de la condition chez le corégone.

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When Smallwood Reservoir, Churchill Falls, Labrador, became operational in 1974, vast numbers of lake whitefish (*Coregonus clupeaformis*) were observed congregating below the Lobstick control structure (Fig. 1). Attention was drawn to these fish when substantial numbers were killed or injured by hydraulic forces generated during reopening of the control gates (flow capacities range from 0 to 3400 $m^3 \cdot s^{-1}$). Initial studies of the fish-kill problem, conducted by Newfoundland and Labrador Hydro and the Churchill Falls (Labrador) Company CF(L) Co, suggested that whitefish may remain in the vicinity of the structure for long periods and may become thin and emaciated (Ferguson 1974; Bishop 1976; and pers. obs.).

Bodaly et al. (1984) have reported a similar occurrence involving lake whitefish below the Missi Falls control dam, Southern Indian Lake, Manitoba. Since most northern hydro schemes include flow control structures and are in regions where *Coregonus clupeaformis* is common, the situation is of general interest.

The present study describes the impact of a major hydroelectric development in northern Canada on several condition indices of female whitefish. The study compares circuli counts, stomach analyses, and proximate composition analyses of fish of limited size range taken from several areas.

Materials and Methods

Lake whitefish taken immediately downstream of the Lobstick control structure (Lobstick) were compared with fish from Smallwood Reservoir just above the control structure (Reservoir), Ten Mile Lake, a nearby natural lake unaffected by flooding (Ten Mile), and a small number from Jacopie Lake (Jacopie), the east forebay, which experiences flows up to 7000 $m^3 \cdot s^{-1}$. To minimize variation only female fish of fork length 300–450 mm were retained for analysis from samples taken at intervals between June and September. The choice of length range was based on CF(L) Co reports and our observations (M.A.B. and G.P.) which suggested that 350- to 450-mm whitefish were abundant in the reservoir system. This range was extended to 300 mm to allow sampling of Ten Mile fish which were smaller. Mature females occurred at all lengths above 300 mm.

Whitefish from below Lobstick were seined; all others were gillnetted. Samples consisted of 20–25 female fish except the final reservoir sample of 18. Fish were kept in an insulated picnic cooler and chilled by "ice-paks" until examined (usually less than 6 h). Fork lengths were measured to the nearest millimetre and weights to the nearest 0.1 g. Gutted weight is with the gonads, liver, and gut excised.

Livers were weighed (gall bladder removed) and the liver somatic index (LSI) calculated from LSI = liver weight × 100/gutted weight. The ovaries were weighed and the gonad somatic index (GSI) calculated from GSI = gonad weight × 100/gutted weight. The selection of a narrow range of sample sizes precluded any detailed analysis of relationships between organ and body weight; no such relationship was indicated in the samples. Condition factors (CF) were calculated as CF = whole weight (g) × 10^5/length (cm^3). Stomach content values were scored as 1, full; 2, traces of food; 3, empty. Circuli counts record the number of circuli occurring after the last visible annulus on the scale.

The gonads and a portion of the dorsal epaxial muscle were frozen at −11 to −15°C for subsequent proximate composition analysis. Lipid was extracted and determined gravimetrically from 2-g tissue samples according to the procedure of Bligh and Dyer (1959), and protein was estimated in 0.1-g subsamples of tissue using the technique of Lowry et al. (1951). Ash levels were measured by igniting 1.0-g subsamples of tissue for 16 h at 525°C. All constituents are reported as percent wet weight, and all weights are to the nearest 10^{-4} g. Ten fish from each sample were processed for muscle proximate composition analysis; ash levels were measured for 5 fish. Complete analyses were done on gonad samples collected July 9 from Jacopie and September 7–8 from the other sites. Gonad water was measured for all sample periods.

Results and Discussion

Liver somatic index — Different patterns of LSI occurred in each of the three areas (Fig. 2A). LSI's at Lobstick during June through September were significantly lower than those at the other two sites for each sampling period (Duncan's multiple range test, $\alpha = 0.05$). During 1979 there were considerable variations in discharge through Lobstick which were correlated ($r = 0.4612$, $p < 0.05$) with LSI, suggesting that stress increased with flow. LSI's of Ten Mile fish increased steadily; this may be expected in a fall-spawning species over the course of the summer. Smallwood Reservoir LSI's significantly decreased during midsummer (Duncan's multiple range test, $\alpha = 0.05$), which could imply an upstream influence of high discharge through Lobstick during July 1979.

Liver weight or LSI has been correlated with metabolic rate (Medford and Mackay 1978), rate of feeding (Makarova 1973), starvation (Ince and Thorpe 1976), and gonad development

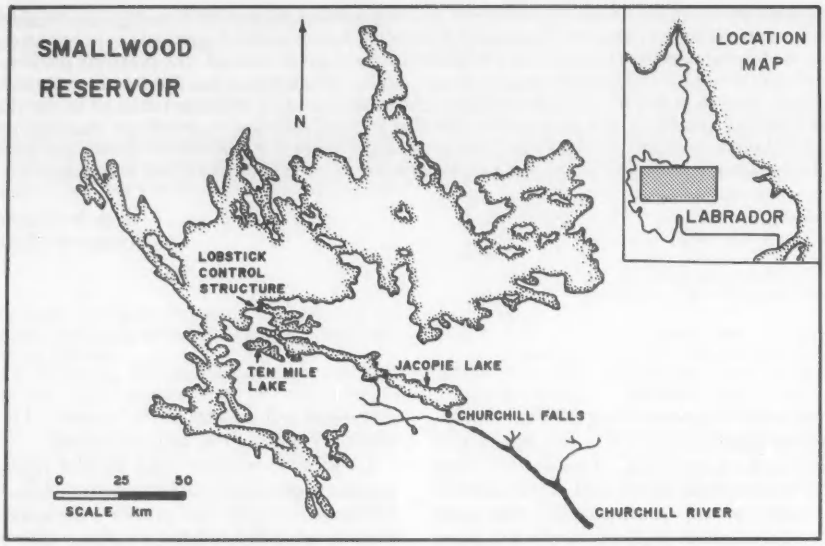

FIG. 1. Smallwood Reservoir with the locations of the Lobstick control structure, Jacobie Lake (east forebay), and Ten Mile Lake, the control lake.

(Reshetnikov et al. 1970). Heidinger and Crawford (1977) used it in assessing environmental impacts; our results suggest this to be an appropriate use.

Gonad somatic index — Fluctuations in GSI closely resembled those of LSI (Fig. 2B). Lobstick fish had the lowest GSI's, which did not increase seasonally. Gonad weight in Ten Mile fish steadily increased and the fish matured normally as the summer progressed. The GSI of the Reservoir fish dropped slightly in midsummer but, unlike LSI values, there was no significant increase towards the end of the summer; rather, the mean GSI's of Reservoir and Lobstick fish were similar in early September (Duncan's multiple range test, $\alpha = 0.05$). Jacopie GSI's were higher than Lobstick, suggesting that the effect of the structure was localized.

Condition factor — There was no significant variation in mean CF in Reservoir and Ten Mile fish during the summer of 1979 (Fig. 2C). Reservoir fish had the highest CF, but Reservoir and Ten Mile fish had similar CF's by early September (Duncan's multiple range test, $\alpha = 0.05$). Lobstick CF's were significantly lower than the other two sites and more variable with no seasonal trend evident and no correlation with discharge or LSI. This agrees with Heidinger and Crawford's (1977) contention that CF is a less sensitive indicator of conditions than LSI. Jacopie fish had high CF's and were not affected by factors influencing Lobstick fish.

Stomach content volume — Stomach content volumes (Table 1) differed between periods (chi-square, $\alpha = 0.05$) for Lobstick and Ten Mile samples. Reservoir volumes were statistically similar at all times. Comparison by area showed significant differences. More Lobstick fish had empty stomachs, especially as the summer progressed, implying that they were not feeding as intensively as whitefish in other areas, including Jacopie.

Circuli counts — Circuli counts have been used to demonstrate seasonal growth (Lee and Power 1976) and frequency of feeding (Bilton and Robins 1971). The number of circuli laid down after the last visible annulus showed that the summer growth patterns may be different at Lobstick compared with the other two sites (Fig. 3). The equations of the three regression lines of mean circuli count (Y) versus time (X = Julian day) were

$$Y = -13.6770 + 0.0912X \text{ (Ten Mile)}, \quad r^2 = 0.058, \quad n = 66$$
$$Y = -19.9563 + 0.1244X \text{ (Reservoir)}, \quad r^2 = 0.63, \quad n = 64$$
$$Y = -3.8096 + 0.0488X \text{ (Lobstick)}, \quad r^2 = 0.31, \quad n = 197.$$

All regression lines are significantly different from each other (ANCOVA, $\alpha < 0.05$). The first circuli were laid down earlier in Lobstick fish and the rate of deposition was slower than in other areas. This may be an effect of continuous flow and turbulence below the control structure, which hastens spring breakup and delays freezing (Baxter and Glaude 1980; and pers. obs.). This allows an earlier onset of feeding in the spring and an extension of feeding later into the fall. However, this only partially compensates for the low rates of feeding and stress associated with the location.

An alternate explanation of the observed results is that fish resident below Lobstick tend not to form a recognizable annulus on the scales; circuli counts early in the summer may be in error, adding previous summer circuli for some fish. We discounted this possibility, which was inconsistent with the regular slow increase in circuli counts observed in the Lobstick fish. Barnes and Power (1984) have, however, reported considerable discrepancies between scale ages and otolith ages in Labrador whitefish, which implies that annuli are not formed regularly on the scales of these fish. Conditions that cause the high spring circuli counts at Lobstick may also have influenced the Jacopie whitefish, since these show elevated counts intermediate between Lobstick and the other two areas.

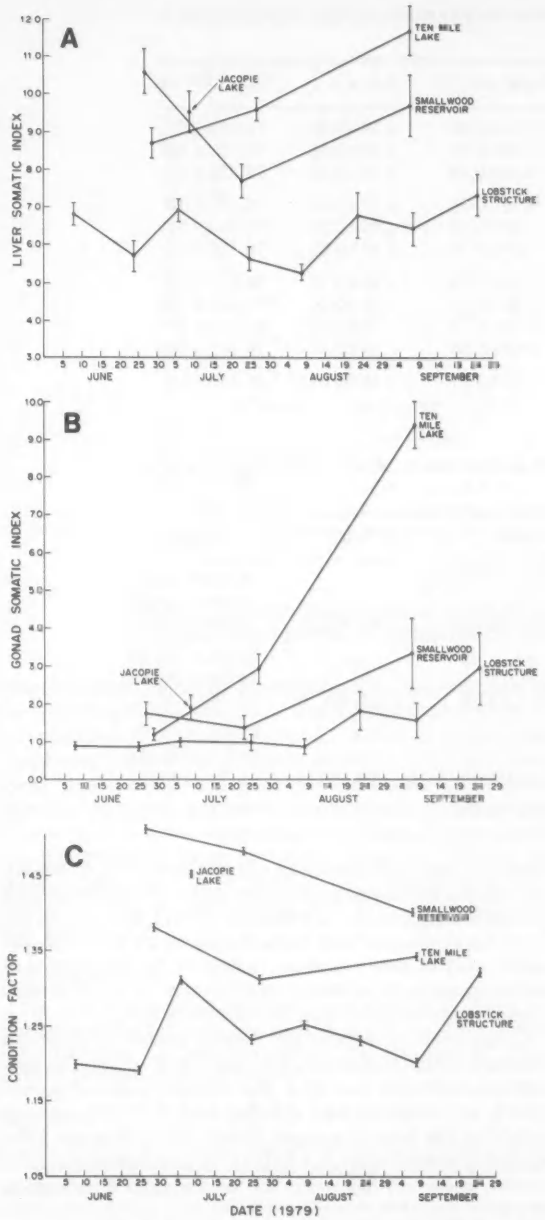

FIG. 2. (A) Liver somatic index, (B) gonad somatic index, and (C) condition factor of lake whitefish from Lobstick control structure, Smallwood Reservoir, and Ten Mile Lake samples during 1979 (mean ± SEM).

Proximate composition — Muscle protein, lipid, ash, and water content varied little with time. Protein levels were similar in Reservoir and Ten Mile fish but significantly lower at Lobstick (Duncan's multiple range test, $\alpha = 0.05$) (Table 2). Muscle lipid was similar in Ten Mile and Lobstick samples and a little higher in the Reservoir; however, lipid levels in Lobstick fish increased from 0.74 to 2.47% between September 8 and 25, the only significant change recorded. The amount of water in the muscle was different for each area (data from all sample times grouped together); Lobstick fish had the most and Ten Mile the least (Duncan's multiple range test, $\alpha = 0.05$). No variation in ash level was evident, by time or area.

TABLE 1. Indices of stomach content volumes for lake whitefish, all sites, 1979 (1, full; 2, food traces; 3, empty).

Site	Date	1	2	3
Lobstick	June 5	2	12	11
	June 24	1	7	17
	July 6	0	4	21
	July 25	0	2	23
	Aug. 8	0	2	23
	Aug. 23	0	7	18
	Sept. 8	0	8	17
	Sept. 25	0	7	18
Reservoir	June 27	12	10	1
	July 23	6	14	5
	Sept. 7	7	9	2
Ten Mile	June 29	8	12	3
	July 27	1	18	2
	Sept. 8	2	17	4
Jacopie	July 9	7	15	0

Gonad water content did not change significantly during the sampling periods in Lobstick fish but steadily decreased in the Ten Mile and Reservoir samples (Table 3). When gonad water contents were pooled from all sampling times, Lobstick fish showed significantly higher values than the other two areas.

Gonad protein levels differed between the various sampling sites in September. Highest amounts occurred in Ten Mile fish, Reservoir values were intermediate, and Lobstick fish yielded the lowest levels (Duncan's multiple range test, $\alpha = 0.05$). Gonad lipid levels were significantly higher in fish from Ten Mile than in those from the other sites. The water content in the gonads of Ten Mile fish decreased during the course of the summer and was significantly lower than in fish from the other areas during September (Duncan's multiple range test, $\alpha = 0.05$). Gonad ash levels did not differ significantly between areas (Table 3).

General Discussion

Anomalies from normal patterns of maturation can indicate stress (Lapin 1973; Lizenko 1973; Bolotova 1976; Craig 1977). In our study, Lobstick fish are clearly stressed; the intermediate condition of the Reservoir fish is clarified when gonad lipid is related to GSI (Fig. 4). The plot shows two groupings: the low-GSI, low-lipid group has a GSI range of 0–1.5, the high-GSI, high-lipid group has GSI values greater than 7.0. This implies that the high group has accumulated sufficient lipid reserves to allow maturation to proceed this year, while the low group has not.

The lack of intermediate values is noteworthy; an individual is in either one group or the other, depending on where it was captured. All but one of the Ten Mile fish (M in Fig. 4) are in the high group. Most Lobstick fish (L) are in the low group. Reservoir fish (R) occur in both the low (2/3) and the high (1/3) groups. This grouping may result from the influence of the structure but more likely it is due to alternate-year spawning. These data agree with those of Reshetnikov et al. (1970) in that a

TABLE 2. Lake whitefish muscle proximate composition analysis results, all sites, 1979, expressed as percentage wet weight (mean ± SEM).

Site	Date	Protein ($n = 10$)	Lipid ($n = 10$)	Ash ($n = 5$)	Water ($n = 10$)
Reservoir	June 27	21.67±2.81	1.24±0.30	1.26±0.09	78.42±1.27
	July 23	19.47±2.49	1.32±0.47	1.29±0.10	78.73±1.58
	Sept. 7	21.46±3.81	1.20±0.34	1.26±0.24	78.48±1.13
Ten Mile	June 29	18.79±3.24	0.98±0.36	1.17±0.14	78.18±1.30
	July 27	21.63±1.55	1.10±0.48	1.45±0.29	75.30±3.54
	Sept. 8	21.17±1.29	1.03±0.20	1.33±0.08	78.24±3.45
Lobstick	July 6	18.62±2.20	1.14±0.78	1.41±0.78	78.67±1.20
	July 25	18.48±3.24	0.95±0.32	1.23±0.09	79.66±1.72
	Sept. 8	19.04±2.77	0.74±0.28	1.29±0.03	81.39±4.08
	Sept. 25[a]	22.29±4.14	2.47±1.33	1.38±0.13	79.48±1.09
Jacopie	July 9	24.05±4.17	1.29±0.27	1.25±0.17	76.37±6.12

[a] $n = 4$ for ash and $n = 9$ for water.

TABLE 3. Lake whitefish ovary proximate composition analysis results, all sites, 1979, expressed as percentage wet weight (mean ± SEM, n in parentheses).

Site	Date	Protein	Lipid	Ash	Water
Reservoir	June 29	—	—	—	76.91±7.34(10)
	July 23	—	—	—	74.96±6.63(10)
	Sept. 7	15.61±4.26(12)	6.92±3.65(18)	1.14±0.17(20)	72.09±8.19(13)
Ten Mile	June 29	—	—	—	81.61±4.76 (9)
	July 27	—	—	—	74.75±5.58(10)
	Sept. 8	19.36±3.03(18)	9.47±1.17(20)	1.01±0.18(20)	65.76±3.16(20)
Lobstick	July 6	—	—	—	79.51±6.12(10)
	July 25	—	—	—	77.71±7.08(10)
	Sept. 8	12.04±3.03(20)	4.83±4.09(19)	1.21±0.18 (8)	78.39±5.92(20)
Jacopie	July 9	12.47±2.67(10)	10.77±6.39(10)	1.09±0.29 (3)	76.37±6.12 (9)

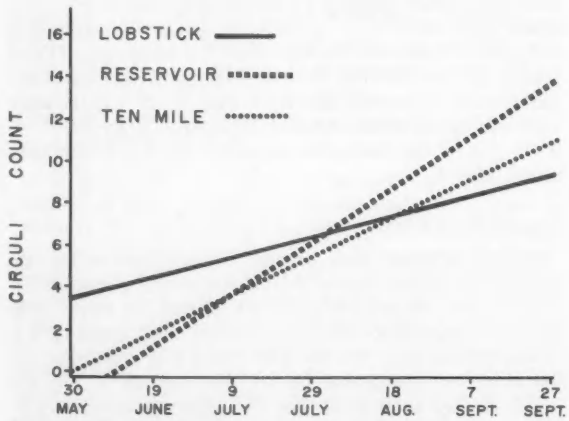

FIG. 3. Numbers of circuli following the last annulus on scales of lake whitefish.

minimum and critical amount of lipid is required for gonad maturation.

Reshetnikov et al. (1970) also noticed that nonspawning whitefish undergo an increase in the amount of muscle lipid in late summer–early fall. This was seen in the sample mean from Lobstick taken on September 25 in which the amount of muscle lipid is 2.47%, up from 0.74% on September 8. This may result from lipid originally allotted to egg production being resorbed and stored in the musculature to serve as an energy reserve. This concept is supported by Shikshabekov (1971), who studied fish trapped in an area of sluice flows. Spawning did not occur, and mature oocytes were resorbed. In light of the apparently low feeding intensity at Lobstick, a similar response would provide a much needed source for the overwintering fish.

Composition of muscle and female gonads of Labrador whitefish conforms generally with published values. The most variable constituent was lipid. The recorded values of percent lipid by wet weight for lake whitefish are 0.2–18.8%, and high variability has been recognized (Dugal 1962; Mangold 1974; Reshetnikov and Yermophin 1975). The constituent analysis of ovarian tissue also fell within the known range for coregonid species.

One interesting difference between Labrador whitefish and those of Reshetnikov et al. (1970) and Bolotova (1976) is that they do not appear to store fat in the body cavity; however, a possible alternative strategy was detected. Liver lipid levels in European whitefish are low, in the range of 1.0–3.0%. We observed 4.3–11.65% in Labrador whitefish. Reshetnikov et al. (1970) noted that the liver can be used as a lipid depot in some salmonids; however, they did not include whitefish. If Labrador whitefish are indeed using the liver as their main lipid depot this would partly explain the sensitivity of LSI to environmental conditions.

The reasons why whitefish reside in what appears to be a very stressful environment below the Lobstick control structure are

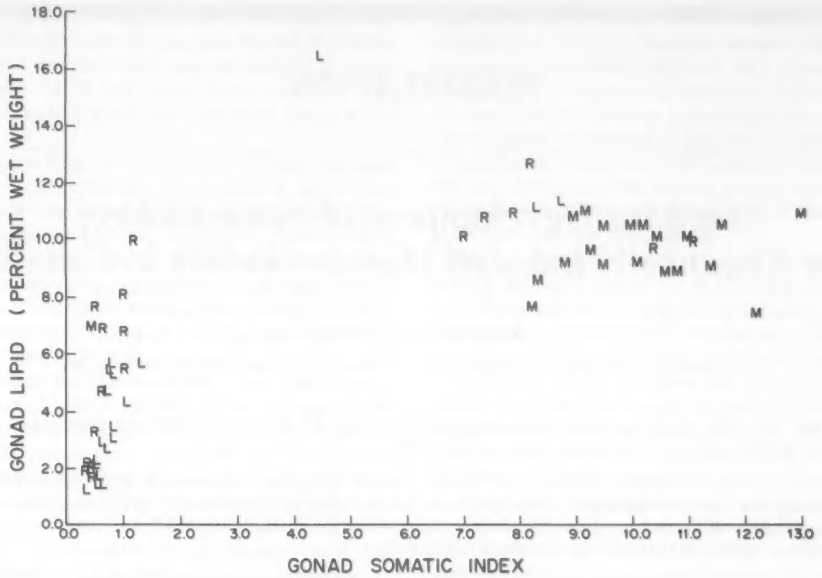

FIG. 4. Relationship between gonad lipid and gonad maturation amongst female lake whitefish from Lobstick control structure (L) Smallwood Reservoir (R), and Ten Mile Lake (M).

difficult to assess. Fish that are swept from the reservoir through the control structure experience a sudden pressure change as they pass from 18 m to near the surface. Evidence from tagging experiments shows that at least some fish remain for several weeks near the structure. The only environmental gradient identified below the structure was a decrease in oxygen content of $0.02\,mg \cdot L^{-1} \cdot km^{-1}$ extending over 80 km. Oxygen levels near the turbulent water are above $12\,mg \cdot L^{-1}$ during the summer. Many fish show evidence of mechanical injuries, particularly after the control gates have been closed, and these may limit mobility.

Acknowledgments

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PERSPECTIVES

Need for Development of Epidemiology for Chemically Induced Diseases in Fish in Canada

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Gilbertson, M. 1984. Need for development of epidemiology for chemically induced diseases in fish in Canada. Can. J. Fish. Aquat. Sci. 41: 1534–1540.

There are few case histories in Canada in which chronic low level exposure to pollutants has been demonstrated to have caused deleterious effects on fish. I challenge the adequacy of approaching aquatic toxicology mainly by laboratory experimentation. I argue that the discipline should start from observations of anomalous biological phenomena in the environment.

Il y a peu de cas répertoriés au Canada où on a démontré qu'une exposition chronique à de faibles teneurs de pollutants avait eu des effets nocifs sur le poisson. Je mets en doute la pertinence d'utiliser l'expérimentation en laboratoire comme approche préférentielle à l'étude de la toxicologie aquatique. Je soutiens que cette discipline devrait être basée sur l'observation des anomalies biologiques dans l'environnement.

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Problems of Investigation for Litigation, Negotiation, and Regulation

During the past 35 yr there has been an increasing involvement of the Canadian Government in the investigation and regulatory control of pollution of the aquatic environment to protect fish, fish habitat, and fisheries. This involvement has its jurisdictional basis in the *British North America Act 1867* and in the *Fisheries Act 1977*. In preparing and enforcing regulations, there has been an implicit premise that damage to fish or fisheries can be demonstrated by measurement of environmental levels of pollutants and extrapolation of the results of controlled toxicity studies or bioassays undertaken using pure compounds or samples of effluent. In addition, any person who lays a charge under the *Fisheries Act* must show that the "deposit" of the "deleterious substance" was into a "water frequented by fish."

This premise has recently come under challenge not only in the courts but also in negotiations with industry on existing and proposed regulations under the *Fisheries Act*. This premise was apparently necessary because it is frequently extremely difficult to observed effects on fish, particularly sublethal effects, in the field. It is even more difficult to demonstrate a causal relationship between the presence of a pollutant and an observed effect. Thus, in the absence of direct evidence of harm, the extrapolation and a reliance on expert witnesses has been necessary as a best approximation.

Does the premise still hold, or have the conditions now changed? There are several examples of instances where this premise has been challenged, and thus the kinds of investigation that government organizations need to undertake to demonstrate causality of damage may need to be reexamined. This challenge has come not only in litigation proceedings under the *Fisheries Act*, Section 33, but also in development of regulations and in international negotiations on acid rain.

In *Regina* vs *Great Canadian Oil Lands Ltd.* in the District Court of Alberta, Edmonton, 1978, the company was acquitted of a charge of permitting the deposit of a deleterious substance in the Athabasca River (MacLatchy 1980). The appeal court upheld the lower court ruling and found that there was no evidence of any harmful effects on the river, nor was there any evidence of deleterious substances in the seepage samples collected from the site despite evidence of acute lethality of the seepage samples to fish under laboratory conditions. Further, the Crown's tests had utilized species of fish not present in the Athabasca River or only rarely so, and these data were deemed insufficient to prove that the seepages were deleterious. This case is of particular interest because it not only shows the necessity for careful work on the environmental pathway whereby the pollutant enters aquatic habitat but also the necessity for information on both effects of the pollutant under laboratory conditions on relevant species and demonstration of actual damage to fish or fisheries. However, in a subsequent case, involving the same company in *R.* vs *Suncor Ltd.*, bioassay results were deemed sufficient to show likely harm to

species in the Athabasca River (MacLatchy 1984). Similarly, in *R.* vs *MacMillan Bloedel (Alberni) Ltd.*, the British Columbia Court of Appeal held that the Crown was not obliged to prove that the water into which the deleterious substance was introduced was rendered deleterious as a result (MacLatchy 1980).

Recent negotiations between the Federal Government and industry concerning amendments to regulations under the *Fisheries Act* concerning metal mining and pulp and paper production have been marked by demands by industry that government should present evidence that industrial operations using existing waste treatment technology are causing deleterious effects on fish or fisheries. When initial regulations were being developed under the *Fisheries Act* during the early 1970s, industry was not under the same economic pressure now imposed by the austerity of the recession. To a large degree, government has been unable to produce sound and persuasive case histories or instances on which further regulations could be promulgated with the concurrence of economically beleaguered industries. It is a moot point whether this lack of case histories is a reflection of the absence of field studies or of the absence of field effects.

The final example concerns acid rain and the preparation of an international agreement on the reduction of sulfur emissions. In work group reports (Anonymous 1983) concerning the *Memorandum of Intent on Transboundary Air Pollution* between the United States and Canada, there was extensive information on the sources of sulfur dioxide emissions, the concentrations in precipitation at various localities, trend data concerning levels in various aquatic habitats, and toxicological data concerning the effects of acidity on fish and fish reproduction under controlled laboratory conditions. The case was, however, relatively weak concerning the incidence of actual damage in the environment and even weaker concerning the prediction of the extent and severity of the likely damage during the next 20–30 yr. A quotation from the report concerning evaluation of the evidence of damage to fish populations in the Adirondack Region of New York captured the then prevailing skepticism of the U.S. administration: "It is difficult to evaluate exactly how many fish populations have been lost from Adirondack waters as a result of acidification. 180 Adirondack ponds that formerly sustained brook trout [*Salvelinus fontinalis*] populations no longer support such populations. It has not however been formally demonstrated that all (or most of) these population extinctions occurred as a result of acidic deposition." No matter what scientists, administrators, or activist groups may think about the politics of using scientific skepticism to avoid imposition of expensive controls, in effect, the quotation has changed the rules of evidence by challenging the original premise. Necessarily it also changes the kinds of work that fish-habitat researchers undertake because laboratory experimentation and chemical analysis of field samples will be insufficient evidence to persuade foreign governments to bring about a nation-wide cleanup.

Formal Demonstration

The argument against having to "formally demonstrate" causality is that in most cases the extent of damage will be unacceptable by the time that the necessary work has been undertaken. There are several examples where causality was inferred or predicted but was insufficient to effect appropriate controls until a formal demonstration was made and a wild population had crashed or was rendered unfit for human consumption. These examples include (1) the widespread extirpation of several predatory bird species brought about by the use of organochlorine insecticides (Hickey 1969), (2) reproductive failure of fish-feeding birds on Lake Ontario likely caused by chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans (Gilbertson 1983), and (3) closure of local inland and marine fisheries because of metals (particularly mercury (Bligh 1970)), polynuclear aromatic hydrocarbons (Uthe 1979), and organochlorine compounds.

The evidence I have so far reviewed has been concerned with existing industrial operations and existing chemicals already in use. A large part of the work on fish toxicology, however, seems to be being undertaken in the laboratory on the premise that we must attempt to predict the likely environmental chemistry and toxicological effects of chemicals newly introduced to commerce so that we can prevent the entry into the environment of those that pose unacceptable hazards. In reality, there are very few new chemicals introduced to Canadian commerce. Of 2000 notifications made persuant to Subsection 4(6) since the *Environmental Contaminants Act* was promulgated in 1976, only about 15 have been new. The rest have been pesticides, dyes, solvents, etc., that have been in use for several years but were being imported or manufactured for the first time by a branch plant company in Canada in a quantity greater than 500 kg. Most of these other compounds had already been subjected to extensive testing in other countries or have been assessed to pose very little hazard to human health or the environment.

Although we have oriented our research to the development of techniques for hazard assessment of these few compounds new to Canadian commerce, we seem to have neglected the far larger problem of chemicals that already exist in the environment. For example, the levels of certain organochlorine compounds in the Great Lakes are among the highest recorded anywhere in the world. Twelve years ago the diversity of compounds in environmental samples was much greater than at present and the levels of individual compounds were about 10 times higher. At that time there were severe reproductive effects documented in birds (International Joint Commission 1982). However, we have barely begun work on the effects of organochlorine compounds on Great Lakes fish and have certainly not "formally demonstrated" any relationship between biological anomalies in wild populations and these chemicals.

What kinds of data are needed to "formally demonstrate" the relationship between an observed effect and a suspected causal agent? National and international agencies frequently meet to produce long lists of the diverse kinds of data needed to assess chemicals and other pollutants. Fortunately, the lists can be simplified into a 2 × 3 matrix (Gilbertson and Brydon 1981). One side of the matrix (Table 1) consists of two simple ideas derived from Paracelsus and classical toxicology: exposure (dose) and effects (toxicity). The second side of the matrix concerns the three distinct kinds of information that provide the evidence for exposure and effects. These are (1) controlled experimental studies, usually undertaken in the laboratory on laboratory animals using individual pollutants, but sometimes undertaken in experimental ponds and lakes, (2) field surveys, sampling, and observations undertaken on wild populations exposed to various environmental pollutants under various uncontrolled environmental conditions, and (3) commercial surveys of the quantities of the pollutant manufactured, used, and released through various routes to the environment.

TABLE 1. Matrix of kinds of studies needed for formal demonstration of toxicological causality.

	Exposure	Effects
Controlled laboratory experimentation	Chemodynamics, pharmacodynamics	Toxicity
Field surveys, sampling, and observations	Environmental levels and trends	Epidemiology (epizootiology) ecosystem health
Sources of release	Quantity and concentration released	Effluent toxicity (bioassay)

Most scientists can relatively rapidly find where their work fits into the matrix. They tend to produce information either on effects or on exposure and tend to work on controlled laboratory experiments or on field surveys or are concerned with effluent analysis of chemical composition or toxicity. The process has now been challenged by the new requirement to produce all six kinds of evidence including field-effects data otherwise known as epidemiology or, for animals, epizootiology. The proportion, however, of epidemiological work being carried out in Canada is miniscule compared with the proportions in the other five categories.

Case Histories

The number of demonstrated cases of pollutants causing subtle and sublethal effects to Canadian fisheries is relatively small. Most cases have been concerned with acute accidental releases such as spills of oil, chlorine, cyanide, or ammonia. In a number of cases, fisheries have been closed because of contamination. For example, there was a closure on Lake St. Clair because of mercury and one on Lake Ontario because of PCB and mirex. But there seem to be few cases in which fisheries biologists were able to show a causal connection between the release of a chemical and effects on the resource through chronic exposure to sublethal concentrations.

Recently I asked the Environmental Secretariat of the National Research Council of Canada if they would prepare a critical review on "The effects of organic industrial chemicals on aquatic organisms in Canadian ecosystems." After some time I was contacted and informed that there were very few cases of effects on aquatic organisms. The few case histories in Canada included (1) widespread mortality and reproductive failure among Atlantic salmon (*Salmo salar*) in the 1950s caused by the use of DDT in forestry (Kerswill 1967), (2) reproductive failure in the late 1960s in ranch mink (*Mustela vison*) fed Pacific salmon (*Oncorhynchus kisutch*) from the Great Lakes that contained high levels of various organochlorine compounds (Gilbert 1969; Aulerich et al. 1973), (3) thinning and consequent breakage of eggshells of fish-feeding birds throughout North America as a result of DDT contamination (Anderson and Hickey 1972), and (4) an outbreak of chick-edema disease in Lake Ontario fish-feeding birds in the early 1970s, likely caused mainly by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Gilbertson 1983).

Apart from these four incidents there seem to be no case histories of subtle effects of organic chemicals on aquatic organisms though there are many incidents of major fish kills associated with malfunctioning of pollution control equipment, midnight dumping, and plain ignorance. If we turn to the inorganic compounds, there seem to be even fewer cases. Of course we are beginning to put together a believable case on the effects of acid rain but even at this point it seems to be too uncertain for regulatory action by our neighbors to the south. Apart from this there was an acute outbreak of "red herring disease" in Placentia Bay, Newfoundland, in 1969 caused by elemental phosphorus production (Jangaard 1972). There was also extensive evidence of the effects of the trace metals, zinc and copper, on Atlantic salmon mortality in the northwest Miramichi in the mid- to late 1960s (Pippy and Hare 1969). This latter incident was interesting in that other factors including water temperature and bacterial pathogens were involved. This is one of the few cases in which epidemiological sleuthing, using a multifactorial approach, produced a convincing case history.

Detection and Investigation of Chemically Induced Diseases in Fish

Although there are so few case histories that describe incidents of chemically induced diseases in fish and other aquatic organisms, we are spending a lot of money developing sophisticated biochemical techniques to detect subtle changes in their enzymes. Have we decided that there are no observable chemically induced diseases in wild fish populations and that we should therefore develop methodologies to look for signs of stress as outlined by Selye (1975)? I would contend that the problem does not lie with the lack of sophistication of our techniques but in our approach to the phenomenon of chemicals and fish. I suspect that the reason that we have found virtually no Canadian evidence of fish diseases caused by pollutants is that we do not know what to look for and recognize in the field or how to undertake subsequent etiological research. The result is that we seem to be doing our research the wrong way round. We select an enzyme that might be induced or inhibited by an identified pollutant and determine, in the laboratory, whether the levels are affected by age, sex, breeding condition, feeding regimen, salinity, etc. After we have completed these studies that might take 5 yr and consume scarce resources, we go to the field to find out whether any wild fish populations exhibit markedly elevated or reduced levels that can be correlated with a pollutant. This is then called an "effect" when in reality it may only be a physiological compensatory mechanism in response to the presence of a xenobiotic compound. There is usually no suggestion that this compensation is pathological or related to a disease or how it might be related to stress. In my opinion it would be much more informative first to undertake observations of field populations at various levels of organization, perhaps including biochemical, to see whether any anomalies are occurring. Some of these observations of field populations should be undertaken in the laboratory to overcome (1) the inherent problems of observing fish in the field and (2) the problems of disappearance of diseased fish due to predation. If some anomalies were occurring and plausible hypotheses could be formulated, it would then be worth spending resources on laboratory experimentation and developing further biochemical and histological methods. At present there are few observed anomalies and even fewer hypotheses. Thus, most of our research has been oriented towards elaboration of the existing body of knowledge concerning aquatic toxicology (Kuhn 1970)

rather than formulating new principles and posing new avenues of investigation.

A few researchers have used this approach and have found tantalizing evidence that chemically induced fish diseases may be occurring. Outside Halifax Harbour the cod (*Gadus morhua*) have a high incidence of liver pathological states (Freeman et al. 1983). They also have some PCB present but the research is a long way from "formally demonstrating" a causal connection. In the Great Lakes there is a high incidence of tumors in several fish species (Sonstegard 1977; V. Cairns, Department of Fisheries and Oceans, Burlington, Ont., pers. comm.), anomalous levels of mixed function oxidase activity (R. F. Addison, Bedford Institute of Oceanography, Dartmouth, N.S., pers. comm.), and thyroid hyperplasia in coho salmon (*Oncorhynchus kisutch*) (Sonstegard and Leatherland 1976). There is also a myriad of organochlorine, polynuclear aromatic hydrocarbon, and pesticide compounds present, but again the demonstration of causality has proved extremely elusive. Similarly, there are obervations of tumors in lemon sole (*Parophrys vetulus*) samples collected close to urban discharges from Vancouver (Stich and Acton 1976).

I believe we have excessively concentrated our efforts on development of oversophisticated biochemical techniques that are not specifically diagnostic of particular chemically induced diseases, i.e. they are not pathognomonic techniques. We have consequently neglected to undertake the basic fieldwork to see whether any chemically induced diseases are occurring. Are there any diseases actually occurring? The critical place to look, of course, is in the Great Lakes where, particularly 10 yr ago, there were not only the highest levels of individual pollutants such as PCB and dioxin (Gilbertson 1983) but also the greatest diversity of persistent and biologically active compounds. But it is rather late to start looking for effects, even in Great Lakes biota, because the levels are now very low compared with the levels in the past. If they were not overt then, it is unlikely that anything can be seen now.

I suspect there might be or might have been an outbreak of the disease (Helder 1980) analogous to chick-edema disease, in fish in the Niagara River and Lake Ontario caused by chlorinated dibenzo-*p*-dioxins. I am not sure that there are any other locations in Canada where organic pollutants are at sufficient concentrations to cause diseases. Even if they are occurring, we will not find them by confining our work to our laboratories and by neglecting to develop an epidemiological approach. It might be worth looking for shell erosion in crustacea from Sydney Harbour, Nova Scotia, because of coking operations or fin rot in fish from Belledune, New Brunswick, because of industrial wastes. In addition, we should be listening to the observations of the fishermen themselves to obtain an initial warning that something anomalous, that might be related to chemical pollutants, is occurring. For years it has been known that pike (*Esox lucius*) caught near Maitland, on the St. Lawrence River, had an anomalous incidence of scoliosis. Recently, P. Hodson (Department of Fisheries and Oceans, Burlington, Ont., pers. comm.), while investigating lead levels in fish sampled at Maitland, listened to these anecdotes and realized that the skeletal anomalies were not caused by an intrinsic factor such as genetics, as was commonly thought, but by an extrinsic factor such as contamination by organometallic compounds.

Let me take an analogy from medicine. In 1876, Koch isolated the anthrax bacillus from diseased domestic animals, cultured the microorganism, and was able to infect healthy mice and produce the characteristic symptoms of the disease. The analogy is rather informative for understanding what aquatic toxicologists are doing. Koch did not start his investigations with a bacillus or an array of microbes; he started it with a disease. In contrast, aquatic toxicologists seem to have started with an array of chemicals instead of starting with an observation of a disease. Most chemicals, like most microorganisms, are innocuous. Only a very small proportion are capable of causing disease and injury. By not starting with a disease, aquatic toxicologists have not focussed their work on critical compounds and pathways and thus have been unable to contribute very much except to say which compounds are very toxic and should not be put into the aquatic environment at more than a certain concentration.

Koch identified the criteria that were required to "formally demonstrate" the causal relationship between a specific microorganism and a specific disease. These criteria became known as Koch's postulates and were as follows: (1) The microorganism must be present in every case of the disease, (2) the microorganism must be isolated from the diseased host and grown in pure culture, (3) the specific disease must be reproduced when a pure culture of the microorganism is inoculated into a healthy susceptible host, and (4) the microorganism must be recoverable once again from the experimentally infected host.

We can formulate an analogous set of criteria for investigations of any diseases that we suspect are caused by chemicals. We could synthesize the suspected causal compound or use preparative methodologies to isolate the compound instead of culturing the microorganism.

I think that most scientists are, not unnaturally, daunted by the complexity of the multifactorial agents that might be involved in chemical epizootiological work on natural populations of fish. Most have decided to work in the natural laboratory where they can produce unequivocal results using gentically known strains of laboratory organisms exposed to pure compounds under controlled laboratory conditions. This predictably produces publications of a high quality but it does not advance our knowledge of what is actually occurring in the environment. As long as government and university scientists, activist groups, and politicians are up against industry, both here in Canada and in other nations, this is not going to be sufficient.

Science at the Legal and Political Interfaces

In 1976 and 1977 I led government Task Forces in the development of scientific cases on which to regulate PCB (Environment Canada/Health and Welfare Canada 1976) and mirex (Fisheries and Environment Canada/Health and Welfare Canada 1977) under the *Environment Contaminants Act*. In both cases there was evidence concerning the levels of these compounds in various materials from the aquatic environment but there was no evidence that these levels were of any toxicological significance to fish. To a large extent, PCBs and mirex were regulated on the basis of the public concern generated by the media. If a company had objected to the quality of the evidence against PCB, the government would have found it difficult, based on the available scientific evidence, to convince a Board of Review that there was a significant danger from these compounds. These situations have made me extremely conscious of the question of what kinds of information are required for the justification for the production of regulations. If there is evidence of harm in laboratory tests, is there a need to show that the same harm is occurring in the field?

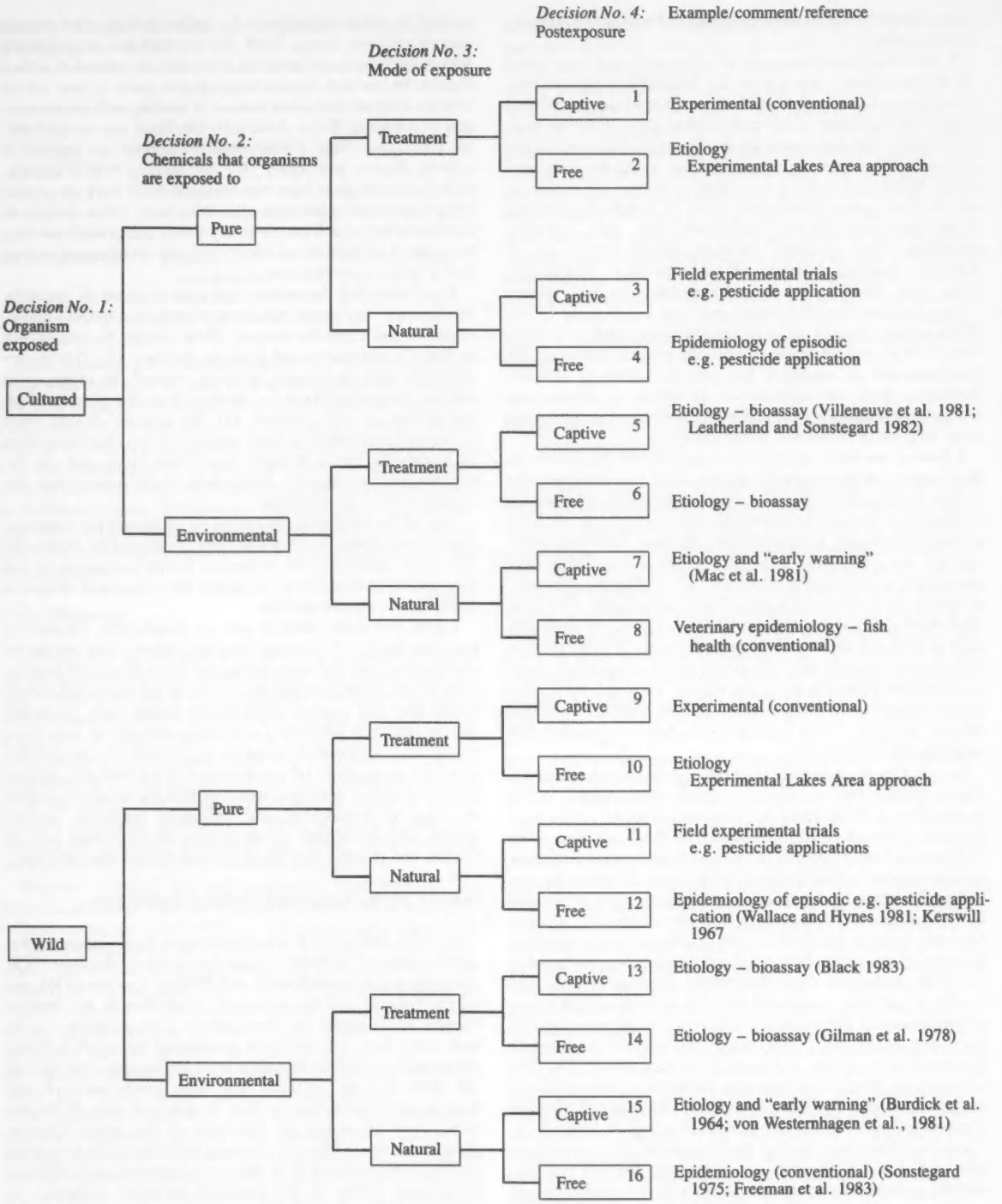

FIG. 1. Decision tree for designs of aquatic toxicology investigations.

Do we need to establish the government case when developing regulations, in a manner that is comparable with the quality of information required for litigation?

The Cabinet Directive, concerning the socioeconomic impact assessment for regulations, concerning health, safety, and fairness involving more than $10 million, requires the Federal Government to gather data on likely benefits of the proposed regulation. This requirement means that we need a measure of the actual damage and of the likely increases in fish production that will accrue from implementation of the regulations. How can we ensure that this information for regulatory decision making is prepared in a timely manner and that it is reliable?

During the past decade several regulations have been promulgated under the *Fisheries Act* to bring about at least minimum treatment of effluents in selected industrial sectors. Despite the expenditure of substantial amounts of money we have little idea as to whether it has benefited fish, fish habitat, or man's use of fish. Investigations to demonstrate long-term trends in fish populations and their health are extremely difficult. To demonstrate reliably a causal relationship with a pollutant is even more difficult. However, negotiations with industry have shown that, if the Federal Government wishes to impose further regulations under the *Fisheries Act*, it must prepare a regulatory case that can reliably demonstrate that discharges are causing a deleterious effect on fish, fish habitat, or man's use of fish and that proposed remedial action would produce a tangible benefit.

Prescription

So how should we proceed? First, we have to become naturalists who know what nature looks like normally. We should take at least about 5% of our time looking in the field for "early warning" signs of biological anomalies such as reproductive failure, growth changes, excess mortality, or lumps and bumps. This search for biological anomalies is a prerequisite for meaningful aquatic toxicology. I suspect, however, that most aquatic toxicologists spend no time looking for these field clues. This is not work that requires sophisticated equipment or techniques but needs sophisticated senses and knowledge. Some of this "field" work can even be undertaken in the laboratory to overcome the inherent problems of field observation. These are not experiments because there are no treatments, merely observations to see whether anything is happening. Second, if we do find something, we should formulate hypotheses that can be tested experimentally in the laboratory. Third, we should start designing some unconventional experiments and ways of observing and finally go back to the field to see whether we have in fact managed to mimic the field situation in the laboratory.

I should explain my call for unconventional experiments and ways of observing. It seems to me that the design of all aquatic toxicology, whether field observational or laboratory experimental, is made up of only four basic design decisions. The first decision is whether we are going to treat or observe cultured or wild specimens. The second decision concerns the chemicals to which the specimens will be exposed. Will these be "pure" chemicals from industry or off the laboratory shelf or will they be chemicals in or from the environment? The third decision is how the organisms will be exposed. Will they be treated experimentally by feeding, dermal application, or by injection, or will they be exposed by "natural" uptake from the environment? The final decision concerns where they will live (or die) after exposure: captive in a laboratory, under controlled conditions, or free in the field. These binary decisions lead to 16 quite separate experimental or observational designs. Figure 1 shows diagramatically how these decisions yield the 16 designs and includes some references to studies that have been conducted using some of these. The order of the various designs yields a continuum from the completely laboratory experimental to the purely naturalist approaches. Most aquatic toxicologists stay with one conventional design because it will safely produce publishable results. Unconventional designs, however, will yield new insights and will likely produce more valuable evidence that can be used to build a solid regulatory case by corroborating evidence from the more conventional approaches. No one approach is very persuasive. However, a combination of approaches is a much more potent and reliable strategy for assembling information on which pollution control decisions can be formulated and made. Though aquatic toxicology in Canada has developed extensively during the past 10 yr, it is still experiencing a crisis of epistemology (Ziman 1979) in contrast with mammalian toxicology, which is a more mature science. This crisis is the basis of many of the frustrations of trying to provide sufficient, relevant, and reliable information for regulatory action.

To make a complete and well-balanced scientific case on which rational action can be based, there is a priority requirement for the development of a symbiotic relationship between laboratory experimentalists and field observers, preferably working as problem-oriented teams prepared to use unconventional approaches. Too frequently in the past there has been an antipathy between the two conventional approaches; one group was accused of lacking scientific rigor, the other of lacking pragmatism and applicability of their results. Generally, however, the conventional experimentalists have won out with the consequence that there is an enormous literature on what pollutants do but almost nothing on what they have done.

It is time to put on the SCUBA gear, sou'wester, and waders and to go to severely polluted aquatic habitats to observe what is actually happening to fish in the real world. It is no longer enough to document exposure and experimental effects; we have now been forced to formally demonstrate effects in the environment. Only after we have recognized a biological anomaly and formed an hypothesis can we return to the laboratory to undertake toxicological research and investigate its etiology and to bring about controls that are based on rigorous science rather than on polemics.

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BOOK REVIEWS/COMPTES RENDUS

Lakes of the Warm Belt. *Colette Serruya and Utsa Pollingher. Cambridge University Press, Cambridge. 569 p. 1983. $89.50 (U.S.)*

The Warm Belt extends over the areas of the earth, approximately between 30°N and 30°S, that have not experienced any marked cold period since the end of the Palaeozoic. Although large variations in humidity have occurred in many regions of the Warm Belt, the dominating, uninterrupted warm climate has persisted, even during the catastrophic events of Pleistocene glaciation. In the absence of continental glaciation, a primary force of lake formation, lakes of the Warm Belt are relatively sparse. The area experiences small seasonal variations in temperature, whether in hot lowland regions or in cold areas of high elevation. From irradiation energy balance considerations, the Warm Belt is the portion of the earth where the net all-wave radiance balance of the earth–atmosphere complex is positive.

Serruya and Pollingher have provided a detailed summary of salient physical, chemical, and biological characteristics of the primary lakes and rivers of South America, Central America, Africa, the Middle East, southeastern Asia, and Australia. This systematic description, occuping some 78% of the book, evaluates the limited knowledge of major lake histories, hydrologies, hydrodynamics, chemistry, and biology. Although tedious and redundant in presentation, the geology and chemistry of the different regions of the Warm Belt are so different and varied that interest is maintained amongst the many lists of dominant flora and fauna by the authors' attempts to interpret the observed major differences in productivity and biotic diversity.

By far the most interesting and instructive portions of the book are the brief, concise discussions of the general historical geological, hydrological, and climatic features of the first three chapters, and the concluding summarizations on water circulation patterns, chemistry, biological diversity, and food webs. Nearly all of the information on lakes from these regions is in a fledgling descriptive phase. Notable exceptions exist and these more comprehensive studies, combined with the extensive functional knowledge from temperate limnology, were used to generalize, often by implication, on probable interrelationships. Although many of the biotic actors of the Warm Belt differ from temperate and arctic regions, the functional ecological play is similar among all.

Certain characteristics of Warm Belt lakes, however, are specific to these regions, and emphasis was given to them. Even though the seasonal variations in temperature are small, precipitation and prevailing wind velocities exhibit distinct seasonality which impacts thermal stabilities and circulation patterns, nutrient loadings, dilution, and distribution, phytoplankton development and succession, and zooplankton and fish reproductive patterns. Thus, although the authors correctly emphasize the importance of aperiodic meteorological phenomena on the lake dynamics in the Warm Belt, they are certainly no more extreme than in the temperate zone. The important points are that they exist and impart a distinct chemical and biotic seasonality.

The compilation of information on biota of lakes of the Warm Belt from many diverse, scattered sources alters common preconceptions about limited biotic diversity, particularly among the algae and zooplankton. Algal diversity is as great or greater than in the temperate zone. Although algal biomass and its variability, often less than one order of magnitude seasonally, are low, primary productivity is high. The causes of the paucity of limnetic Cladocera, in contrast with other zooplankton, and restrictions to those species of small body sizes are poorly understood; research on predator–prey and feeding specificity relationships offers much promise in the future. The extensively studied high species diversity of fishes in lakes of the Warm Belt is summarized. Although no new insights are offered, the diversity is abundantly substantiated by the many examples discussed.

The two gaping voids in contemporary limnology are the functional interrelationships and controls of bacterial degradation of particulate and dissolved organic matter and the coupling of metabolism of littoral flora to pelagial carbon cycling and nutrient dynamics. That ignorance is even more acute among lakes of the Warm Belt. Less than a dozen of the hundreds of references cited address bacterial metabolism. The authors correctly avoid generalization on quantitative integration of carbon cycling and food web analyses. Instead, four divergent types of lake food webs are summarized in a classical approach. The dominance of aquatic macrophyte and allochthonous loadings of organic matter and detrital pathways of metabolism are overwhelming in many lakes and rivers of humid regions. Planktonic dominance emerges in large lakes of arid regions, but the presumptions of the importance of zooplankton in nutrient remineralization continue to be made without quantification of bacterial degradation rates and functions in nutrient cycling. Until this admitted "missing link" is fully understood in quantitative terms, the present incongruities of imbalances in carbon budgets and nutrient fluxes cannot be resolved.

Serruya and Pollingher also discuss a number of large reservoirs that have been created in the Warm Belt. In example after example with even the most conservative evaluations, it is obvious that the many deleterious effects (e.g. altered hydrology, disease, macrophyte explosions, alterations and losses of species diversity, etc.) often outweigh the advances (e.g. electrical energy) that are accrued. It is clear that reservoir

construction in the tropics must be done with much more rigorous evaluations of the *net* benefits to the developing countries than has been true in the past.

The authors have contributed extensively to advancement of limnological interest in the lakes of the Warm Belt by means of this compilation and evaluation. Limnologists of the industrialized temperate region, where most efforts are directed to controlling productivity of surfaces waters for water supply and recreational uses, should know this book for the differences in both the types of lakes in the Warm Belt and their primary exploitation to maximize productivity as a protein resource. It is highly recommended reading.

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ANNOUNCEMENTS/AVIS

The Second International Symposium on Genetics in Aquaculture will be held June 23–28, 1985, in Davis, California. The aim of the symposium is to provide a forum for the exchange of scientific information on all aspects of genetics of aquatic organisms. The scope of discussion is limited to species currently being cultivated, or which have the potential for cultivation. Participation is invited from scientists and aquaculturists interested in genetics and breeding.

For further information, contact Graham A. E. Gall, Second Symposium, Department of Animal Science, University of California, Davis, CA 95616, USA. Telephone: (916) 752-1257; TWX 910-531-0785.

La deuxième colloque international sur la génétique en aquiculture aura lieu du 23 au 28 juin 1985 à Davis (Californie). L'objet du colloque est de fournir une tribune pour l'échange d'information scientifique sur tous les aspects de la génétique des organismes aquatiques. Les discussions ne porteront que sur les espèces faisant actuellement l'objet d'un élevage ou qui possèdent un tel potentiel. Les scientifiques et aquiculteurs intéressés à la génétique et à la reproduction sont invités à y participer.

Pour de plus amples informations, veuillez contacter Graham A. E. Gall, Second Symposium, Department of Animal Science, University of California, Davis, CA 95615, USA. Téléphone : (916) 752-1257; TWX 910-531-0785.

Third International Conference on Toxic Dinoflagellates

The Third International Conference on Toxic Dinoflagellates will be held June 8–12, 1985, in St. Andrews, New Brunswick, Canada.

Sessions, chairpersons, and invited speakers include:
Keynote Address: J. C. Medcof
Organisms: K. A. Steidinger, F. J. R. Taylor
Environments: D. M. Anderson, P. M. Holligan
Chemistry and Biochemistry: Y. Shimizu, T. Yasumoto
Toxinology and Pharmacology: D. G. Baden, W. A. Catterall

A limited number of contributed papers, to be solicited in the fall of 1984, will be included in each session. Emphasis will be placed on a poster session, on practical workshops (sampling, culturing, taxonomy, toxin analysis and assay, etc.), and on a panel discussion/workshop on management in fisheries and public health problems associated with toxic dinoflagellates.

To receive the next announcement, contact Alan W. White, Conference Chairman, St. Andrews Biological Station, Fisheries Research Branch, Department of Fisheries and Oceans, St. Andrews, N.B., Canada E0G 2X0. Telephone: (506) 529-8854.

Steering Committee:	International Liaison:
B. C. Abbott	E. Balech
D. M. Anderson	M. Estrada
D. G. Baden	J. L. Maclean
Y. Shimizu	T. Okaichi
A. W. White	K. Tangen
C. M. Yentsch	

Troisième conférence internationale sur les dinoflagellés toxiques

La troisième conférence internationale sue les dinoflagellés toxiques aura lieu des 8 au 12 juin 1985 à St-Andrews (Nouveau-Brunswick), Canada.

Sessions, présidents et conférenciers invités inclus :
Allocution dominante : J. C. Medcof
Organismes : K. A. Steidinger, F. J. R. Taylor
Environments : D. M. Anderson, P. M. Holligan
Chimie et biochimie : Y. Shimizu, T. Yasumoto
Toxinology et pharmacologie : D. G. Baden, W. A. Catterall

Un nombre limité de contribution d'articles, sollicités à l'automne de 1984, seront inclus dans chaque session. L'emphase sera placée sur la session d'affichage, sur le cercle d'étude pratique (échantillonnage, culture, taxonomie, anlayse et essais des toxines, etc.) et sur un groupe de discussion sur les problèmes de la gestion des pêches et de la santé publique associés avec les dinoflagellés toxiques.

Pour recevoir le prochain avis, contacter Alan W. White, Président de la conférence, Station de biologie de St-Andrews, Direction de la Recherche sur les Pêches, Ministère des Pêches et des Océans, St-Andrews (N.-B.), Canada E0G 2X0. Téléphone: (506) 529-8854.

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COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Articles

Cost of sustained and burst swimming to juvenile coho salmon (*Oncorhynchus kisutch*) **K. J. Puckett and L. M. Dill**

Larval cohort succession in herring (*Clupea harengus*) and capelin (*Mallotus villosus*) **T. C. Lambert**

Reproductive strategies of demersal and pelagic spawning fish **T. C. Lambert and D. M. Ware**

Uptake and accumulation of inorganic tin by a freshwater alga, *Ankistrodesmus falcatus* **P. T. S. Wong, R. J. Maguire, Y. K. Chau, and O. Kramar**

Changes in elemental composition of hatchery-reared rainbow trout, *Salmo gairdneri*, associated with growth and reproduction **K. D. Shearer**

Multistage sampling designs in fisheries research: applications in small streams **D. G. Hankin**

Extraction of algal ATP and interpretation of measurements **L. L. Hendzel and F. P. Healey**

Importance to mussels of the benthic boundary layer **D. J. Wildish and D. D. Kristmanson**

Phosphate exchange between littoral sediments and overlying water in an oligotrophic north-temperate lake **A. J. Twinch and R. H. Peters**

Short-term colonization patterns of lotic macroinvertebrates **J. J. H. Ciborowski and H. F. Clifford**

Some causes of variation in estimates of mean weights for *Hydropsyche* larvae (Trichoptera: Hydropsychidae) **R. J. Mackay**

Quantification of wood habitat in subtropical Coastal Plain streams **J. B. Wallace and A. C. Benke**

Nearshore ichthyoplankton of a north temperate estuary **H. Powles, F. Auger, and G. J. FitzGerald**

Relative importance of anthropogenic versus natural sources of acidity in lakes and streams of central Ontario **B. D. LaZerte and P. J. Dillon**

Electrolyte and water balance in plasma and urine of rainbow trout (*Salmo gairdneri*) during chronic exposure to cadmium **M. A. Giles**

Osmoregularity and hematological responses of rainbow trout (*Salmo gairdneri*) to extended environmental acidification **M. A. Giles, H. S. Majewski, and B. Hobden**

Changes in populations and drift of stream invertebrates following lampricide treatment **R. M. Dermott and H. J. Spence**

Phosphorus flux through epilimnetic zooplankton from Lake Ontario: relationship with body size and significance to phytoplankton **W. D. Taylor**

Notes

Uptake efficiency of the gills of English sole (*Parophrys vetulus*) for four phthalate esters **B. L. Boese**

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Scombrids of the world (Bruce B. Collette and Cornelia E. Nauen) **W. B. Scott**

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